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Applying the isotope pairing technique to evaluate how water temperature and habitat type influence denitrification estimates in Breton Sound, Louisiana

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APPLYING THE ISOTOPE PAIRING TECHNIQUE TO EVALUATE HOW WATER TEMPERATURE AND HABITAT TYPE INFLUENCE DENITRIFICATION ESTIMATES IN BRETON SOUND, LOUISIANA

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ABSTRACT

The upper Breton Sound estuary was hydrologically reconnected to the Mississippi River via the Caernarvon freshwater diversion structure in 1991. The Caernarvon structure can provide controlled freshwater pulses to the upper Breton Sound estuarine ecosystem, replicating historic freshwater pulsed events, although the original authorization was to control salinity isohalines at specific locations in the estuary. However, unlike historic freshwater pulsed events prior to the construction of levees, the current freshwater pulse contains an unprecedented amount of inorganic nitrogen, predominately as nitrate (annual average 71.4 μM NO_3^-). Denitrification is a microbial process, which can potentially remove excess nitrate entering coastal Louisiana ecosystems due to these riverine pulsed events. This study presents the first ^{15}N isotope pairing technique (IPT) denitrification estimates from coastal sediments in Louisiana, and evaluates the influence water temperature and different habitat types have on denitrification rates. Three IPT assumptions were tested in the current study and were fulfilled; however, the fourth assumption, the influence of annamox, was not evaluated. The three IPT assumptions evaluated were fulfilled in the benthic and marsh habitat sediments. However, the marsh habitat sediments from upper Breton Sound provide a dilemma for the current IPT design; $^{15}NO_3^-$ diffusion will not reach deep into the plant rhizosphere where optimal conditions persist for coupled nitrification-denitrification activity. There was a significant interaction between habitat (marsh and benthic) and water temperature (8 and 22 °C) treatments on denitrification rates. Mean total denitrification (direct + coupled denitrification) estimates from a 70 μM $^{15}NO_3^-$ incubation concentration were 17.5 (± 3.1) and 5.1 (± 1.5) μmol N m^{-2} h^{-1} for benthic and marsh habitat sediments at 22 °C, and were 7.8 (± 1.9) and 2.1 (± 0.45) μmol N m^{-2} h^{-1} for benthic and marsh

habitat sediments at 8 °C, respectively. Overall, total denitrification rates ranged from 0.28 to 284.1 μmol N m^{-2} h^{-1} for both habitats at 22 °C over a 2 to 200 μM $^{15}NO_3^-$ incubation concentration range. Coupled denitrification comprised the majority of the total denitrification rate measured. Mean direct denitrification rates did not exceed 2 μmol N m^{-2} h^{-1}, and suggests direct denitrification measured by the isotope pairing technique in my study is not a major pathway for NO_3^- removal in upper Breton Sound benthic and marsh habitats.

CHAPTER 1. INTRODUCTION

An Overview

The geomorphology, ecology and biogeochemistry of coastal Louisiana are largely driven by the freshwater, sediment, and nutrients delivered from the Mississippi River. The Mississippi River oscillated over coastal Louisiana's landscape creating a vast deltaic system of wetland habitats over the Holocene epoch. Upon European occupation in the early 1700's, the variability in Mississippi River flooding created challenges for establishing permanent settlements along the river and ideas of "containing" the river for flood control began to emerge. However, large-scale Mississippi River containment throughout the Mississippi River basin was not mandated by the federal government until after the great Mississippi River flood of 1927 (Barry 1997). By 1928 the United States Congress established the Flood Control Act of 1928, which mandated the U.S. Army Corps of Engineers and the Mississippi River Commission to prevent future flooding disasters on the Mississippi River for the sake of national prosperity (Barry 1997). By the mid-nineteenth century levees lined the Mississippi River from Cairo, Illinois to the Gulf of Mexico. This eliminated the natural river oscillations and overbank flooding in the Mississippi deltaic plain, rather conveying the freshwater, sediments and nutrients directly into the Gulf of Mexico. This eliminated the freshwater and sediment supply to coastal Louisiana deltaic wetlands, which in turn, accelerated the loss of these wetlands; currently disappearing with estimates ranging from 17 to 35 square miles per year (Fischetti 2001; Barras et al. 2003; USACOE 2006). Coupled with this drastic land loss is the development of expansive hypoxic areas, which is driven by excessive nutrient inputs derived from agricultural operations

in the Mississippi River watershed, during summer seasons in the Gulf of Mexico (Turner et al. 2005; Turner et al. 2008).

Mississippi River diversions (such as Caernarvon diversion) were built in coastal Louisiana to increase fish and wildlife productivity by re-constituting favorable salinity conditions in many remnant Mississippi River basins, such as Breton Sound basin (USACOE 2006). More recently, coastal Louisiana has considered using Mississippi River diversions as a restoration strategy to prevent, or slow further land loss, and to abate the hypoxic area which annually develops in the Gulf of Mexico (Mitsch et al. 2001; Delaune and Jugsujinda 2003; Delaune et al. 2003; Wheelock 2003; Mitsch et al. 2005). This could be accomplished by using Mississippi River diversion structures; albeit with modifications to their initial authorized use to re-establish favorable salinity conditions. Instead, river diversion operation should mimic historic flooding events, or freshwater pulses, which divert large volumes of freshwater and sediment from the Mississippi River into adjacent coastal wetlands and water bodies. However, with the freshwater and sediment come nutrients, especially nitrate (NO_3^-), which travel down from the upper Mississippi River watershed to the lower reaches of the river in high concentrations (annual average 71.4 µM NO_3^-) creating ecological, economic and social concerns about these engineered diversions (Lane et al. 1999; Battaglin et al. 2001; Goolsby and Battaglin 2001; Swarzenski et al. 2008). Coastal Louisiana scientists, land managers and government officials are challenged with defining the benefits of freshwater and sediment inputs to ecosystem restoration, in conflict with the negative consequences of high NO_3^- inputs to coastal waters that may impair water quality. Strategies to reduce these high NO_3^- concentrations, and a better understanding of how NO_3^- cycles through coastal Louisiana wetlands, is needed to implement and establish the design and operations of freshwater diversion

structures within the Mississippi River delta. One important mechanism for removing high NO_3^- concentrations in coastal Louisiana could be denitrification. However, the magnitude by which denitrification may alleviate high NO_3^- concentrations, and the environmental conditions and habitat types which enhance the denitrification process are still uncertain, especially in Breton Sound basin in southeastern Louisiana.

The following sections in this chapter provide an overview on the Mississippi River delta, and the biogeochemistry of nitrogen. Narrowing the scope I then transition to provide background on the denitrification process, the isotope pairing technique methodology and to what extent denitrification has been measured in the Mississippi River delta.

The Mississippi River Delta: Geology and Geomorphology

> The process of delta formation depends almost wholly on the following law: the capacity and competence of a stream for the transportation of detritus are increased and diminished by the increase and diminution of the velocity (Gilbert 1885)

The term delta was first noted by the Greek historian Herodotus, about 450 B.C., when he described the triangular alluvial deposit shaped by the Nile River, Egypt (Coleman 1981; Woodroffe 2002). River deltas were fundamental in early civilization development, due to their agricultural production and navigational use. The Mississippi River delta is no exception with archaeological evidence suggesting Native American occupation and cultivation occurring about 2000 B.C. with occupation extending into the eighteenth century. European exploration in the Mississippi delta region first occurred around 1699. Exploration was lead by the French explorist, Pierre Le Moyne d' Iberville. Iberville embarked up-river with a mandate from the French Colony to establish an agricultural trading settlement to compete with the Spanish and English colonies established in Florida and the Carolina's (Kidder 2000).

Generally, deltas can be defined as coastal deposits which consist of both subaqueous and subaerial features fashioned from fluvial sediments and modified by marine processes which include: waves, tides and currents. A delta plain can consist of many different subaerial and subaqueous landforms including: distributary channels, river-mouth bars, tidal flats, interdistributary bays, tidal ridges, beaches, beach ridges, dunes, swamps, marshes and subaerial natural levees (Wright 1978; Coleman 1981; Seybold et al. 2007). The Mississippi River delta includes many different deltaic landforms derived from Holocene materials eroded and transported over thousands of years from the Mississippi River drainage basin.

The Mississippi River and its only active distributary, the Atchafalaya River, has a drainage basin which covers approximately 3,344,560 km^2, an area representing about 41% of the contiguous United States and two Canadian provincial units (Coleman 1981; Coleman 1988; Goolsby and Battaglin 2001). The average Mississippi River discharge at the river mouth is approximately 15,360 $m^3 s^{-1}$ with a range in discharge from 2,830-60,000 $m^3 s^{-1}$ (Coleman 1981). The Mississippi River annual sediment load averages 6.2 x 10^{11} kg (Coleman 1988).

Around the 15^{th} century and progressing into the early 1900's the Mississippi River was attempting to switch to a more hydrologically efficient course down its major distributary, the Atchafalaya River. The Atchafalaya River offers a shorter outlet to the Gulf of Mexico than the current Mississippi River outlet; approximately 142 miles versus 315 miles, and would have diverted if not for anthropogenic intervention (USACOE 2009). In 1963 the old river control structure was engineered and situated, about 50 miles northwest of Baton Rouge, LA (USACOE 2009), to stabilize the amount of freshwater and sediment captured by the Atchafalaya distributary. The Atchafalaya River was allocated 30% of the combined Mississippi and Red River flow. As a result of this continued freshwater and sediment supply the Atchafalaya River

has been building two bay-head deltas since about 1950; one at the Atchafalaya River Outlet, and the second at the Wax Lake Outlet (Roberts 1997; Woodroffe 2002). Combined with the Red River, the Atchafalaya River annual average discharge is approximately 6500 $m^3 s^{-1}$ with an annual average sediment load of 2.2 x 10^8 kg.

The Mississippi-Atchafalaya combined discharge is about 22,000 $m^3 s^{-1}$ and represents 80% of the estimated freshwater discharge to the Gulf of Mexico (Goolsby and Battaglin 2001). The Mississippi River and Atchafalaya River delta is characteristic of hypopycnal (buoyant) flow, where less dense river water flows out over denser seawater creating a freshwater lens which can extend outward from the Mississippi River mouth for miles (Woodroffe 2002). Due to this large freshwater discharge, and low marine wave, tidal and current energy in the Gulf of Mexico the Mississippi and Atchafalaya are characterized as a river-dominated delta (Roberts 1997).

The Mississippi River delta plain evolved through a delta cycle which originates with a delta-switching event initiated when the Mississippi River switches or diverts course. This occurs from a decrease in the elevation gradient and flow efficiency within the river as its course continues to lengthen during delta progradation. Once the Mississippi River has abandoned a delta complex for a more favorable and efficient river course a different delta complex originates at the new Mississippi River mouth (Roberts 1997; Coleman et al. 1998). These delta-switching events created the vast Mississippi River delta plain and are the foundation for the delta cycle.

There are two major phases in the delta cycle; a constructional (regressive) and destructional (transgressive) phase. The regressive phase has three stages in delta development with the initial construction phase beginning by filling an interdistributary basin (lacustrine) environment. This eventually leads to stage two when a greater water and sediment volume flow

downstream into a shallow coastal environment which initiates bay-head delta development. Since the 1500's, the Atchafalaya River has progressed through this lacustrine-to-bay-head delta development stage. The third and last regressive stage is the continued bay-head delta progradation onto the inner continental shelf. This last regressive delta-building stage is a classic example for the current Balize-birdfoot delta complex (Roberts 1997; Coleman et al. 1998).

Once a delta-switching event occurs and a delta complex has been abandoned by its fluvial source the transgressive delta cycle phase is initiated. There are four transgressive stages in delta destruction with the initial phase being fluvial abandonment of an active delta complex. In stage two the abandoned delta complex is transformed, as a result of marine processes, into an erosional headland, which creates flanking barrier islands. The flanking barrier islands are fabricated from sand sources eroded away from the headland and transported via longshore currents. Continued headland erosion, subsidence and relative sea-level rise lead to barrier island submergence and separation from the mainland headland creating a barrier island arc, which is stage three. The fourth and final transgressive stage is guided by continued relative sea-level rise and overwash processes, which eventually submerges the barrier complex leading to inner-shelf shoal creation. Present day examples from the different transgressive delta stages in Louisiana include: Bayou Lafourche (stage one), the Lafourche delta complex/Caminada-Moreau headland (stage two), Isle Dernieres/Timbalier barrier islands, St. Bernard delta complex/Chandeleur islands (stage three) and Maringouin-Sale Cypremort / Teche delta complex and Ship Shoal (stage four) (Penland and Boyd 1981; Penland et al. 1988; Roberts 1997; Coleman et al. 1998; Woodroffe 2002).

These regressive and transgressive stages in the delta cycle have created six delta complexes across the Mississippi River deltaic plain throughout the Holocene period beginning

about 7500 B.P. (Roberts 1997; Coleman et al. 1998). The six delta complexes built during the Holocene era from oldest to youngest in age are: 1) Maringouin-Sale Cypremort, 2) Teche, 3) St. Bernard, 4) Lafourche, 5) Balize-birdfoot (current Mississippi River delta) and 6) Atchafalaya-Wax Lake. Each delta complex, with the Atchafalaya as the exception, has had a development interval of 1000-2000 years (Roberts 1997). Radiometric dating has confirmed the arrangement and time period for each delta complex (Fisk and Mcfarlan 1955; Frazier 1967; Thornqvist et al. 1996). The six Mississippi River delta complexes created a delta plain larger than the state of New Jersey; covering an area approximately 30,000 km^2 and representing 41% of the coastal wetlands in the contiguous United States (Roberts 1997; Coleman et al. 1998).

Breton Sound is a wetland-estuarine basin, which was created several thousand years ago by the former Plaquemines-St. Bernard delta complex (Scruton 1960; Roberts 1997). Breton Sound has an area of 1100 km^2 and its marsh and estuarine habitats rest on a foundation of fluvial pro-delta clay deposits (Lane et al. 1999; Coleman et al. 1998). Breton Sound is approximately 40 km in length and runs at a southeast angle towards the Gulf of Mexico (Swenson et al. 2006). The estuary is hydrologically contained by the Mississippi River levee system to the west, the spoil banks of the Mississippi River Gulf Outlet to the east, the natural levees of Bayou La Loutre to the north, and is hydrologically open to the Gulf of Mexico at its southern boundary (Lane et al. 1999).

Nitrogen Biogeochemistry

The chemical elements nitrogen (N), carbon (C), phosphorus (P), oxygen (O) and sulfur (S) are all necessary for life sustaining processes. Of these elements, nitrogen is the most abundant in the biosphere, atmosphere and hydrosphere; however, ironic it is the element least

readily obtainable to sustain life processes. For example, in its most common form, dinitrogen (N_2) gas, it comprises 78% of the earth's atmosphere. Dinitrogen is an inert gas and is not readily used biologically due to the activation energy required to break the N_2 triple bond and convert it to reactive nitrogen. Prior to the twentieth century the natural world only had two processes capable of fixing N_2 into reactive nitrogen: lightning and biological nitrogen fixation. As a result, reactive nitrogen was kept in a tight cycle of generation and consumption by organisms at the time. Free living bacteria (*Clostridium*) in symbiotic associations with plants; such as cyanobacteria and leguminous crops, are capable of biological nitrogen fixation. However, around the early 1900's the Germans developed the Haber-Bosch process, an industrial process replicating biological nitrogen fixation, to manufacture nitrogen fertilizers for use in agriculture. The Haber-Bosch process has drastically accelerated the amount of reactive nitrogen entering the terrestrial and aquatic environment, and today poses serious implications for the eutrophication of many ecosystems world-wide (Vitousek et al. 1997; Galloway and Cowling 2002; Rabalais 2002; Galloway et al. 2003; Reddy and Delaune 2008).

All organisms require nitrogen for basic life processes and it is found throughout ecosystems as a complex mixture of organic and inorganic nitrogen compounds. Inorganic nitrogen forms include: N_2, ammonia/ammonium (NH_3/NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), nitric oxide (NO) and nitrous oxide (N_2O). Organic nitrogen contains simple and complex compounds which include: proteins, nucleic acids, amino sugars, urea, genetic material and chlorophyll. Within the aquatic environment, organic nitrogen is typically found in particulate and dissolved form, and inorganic nitrogen is predominantly found in dissolved form. Particulate organic nitrogen is removed from an ecosystem through settling and burial, and dissolved inorganic and organic forms are typically regulated by complex biogeochemical

reactions occurring in the water column or sediment. Inorganic and organic nitrogen compounds can contain nitrogen isotopes with masses which range from 12-18. However, the nitrogen isotopes with mass ^{14}N and ^{15}N are the two most common nitrogen isotopes and are the only two stable forms with natural abundances of 99.64 and 0.36%, respectively. The nitrogen isotopes ^{14}N and ^{15}N are considered important ecologically while the other nitrogen isotopes, ^{12}N, ^{13}N, ^{16}N, ^{17}N and ^{18}N are unstable, radioactive and are not very abundant in nature (Fry 2006; Reddy and Delaune 2008). Nitrogen transformations and cycling between complex inorganic and organic compounds regulates the availability of nitrogen for many different ecological processes.

Nitrogen is unique and sets itself apart ecologically from other major elements (i.e. oxygen, hydrogen, phosphorus, carbon and sulfur) in the biosphere, and especially in wetland/benthic environments, due to its various forms and oxidation states. Nitrogen can be found in wetlands in its most oxidized form, NO_3^- (+5), or in its most reduced form, organic N, NH_4^+ or NH_3 (-3) (Table 1). Several nitrogen cycling reactions in wetland ecosystems result due to these various nitrogen forms and oxidation states. These reactions result in nitrogen transformations between oxidized and reduced forms, and essentially provide electrons for use by microbial organisms in deriving their metabolic energy requirements (Mitsch and Gosselink 2000; Reddy and Delaune 2008).

Nitrogen cycling in wetland and benthic sediments is regulated by oxidation-reduction reactions (redox reactions); a reaction where electrons are transferred between two molecular species causing one species to be reduced (gain electrons) while the other species is oxidized (lose electrons). Redox reactions are unique and depend on the molecular oxygen content within wetland sediments; being performed either in the presence of oxygen (aerobic state) or under low or no oxygen (anaerobic state) conditions. Anaerobic states usually occur when the sediment is

flooded and aerobic states occur when sediments are exposed to the atmosphere. Reduced conditions are especially prevalent in wetland sediments due to fluctuations in water levels, which cause sediments to fluctuate between anaerobic and aerobic conditions. However, microsites of aerobic environments can be present in sediments persistently flooded; found either within the top few millimeters of the sediment surface, due to oxygen diffusion from the overlying water column, or from plant roots within the sediment profile. Wetland plants can transport oxygen down to their root systems where it diffuses into the surrounding soil environment creating a plant rhizosphere. These aerobic-anaerobic zones and microsite environments can create concentration gradients within the soil profile for several different nitrogen species, which results in many different nitrogen transformations (Reddy et al. 1989; Mitsch and Gosselink 2000; Reddy and Delaune 2008).

Table 1 Nitrogen oxidation states, example molecular formulas for each oxidation state and form commonly found or produced in soil (after Reddy and Delaune 2008).

Oxidation State	Molecular Formula	Form in Soil
+5	Nitrate (NO_3^-)	Dissolved
+3	Nitrite (NO_2^-)	Dissolved
+2	Nitric Oxide (NO)	Gas
+1	Nitrous Oxide (N_2O)	Gas or Dissolved
0	Dinitrogen (N_2)	Gas
-1	Hydroxylamine (NH_2OH)	Not Detected
-3	Ammonia/Ammonium (NH_3/NH_4^+)	NH_3-Gas, NH_4^+-Dissolved or fixed on sediments

Nitrogen transformations in wetland sediments are mediated by chemical and biological processes coupled with fluctuations between anaerobic and aerobic conditions (Fig. 1). Ammonification-mineralization, microbial or plant assimilation/uptake and dinitrogen fixation can occur in either aerobic and/or anaerobic conditions. Nitrification is restricted to aerobic

conditions, whereas denitrification and dissimilatory nitrate reduction to ammonium (DNRA) occur only in anaerobic conditions. Ammonia volatilization is controlled by pH and not the oxygen content. Nitrogen ammonification-mineralization is the degradation of organic nitrogen to NH_4^+. Microbial or plant assimilation/uptake is the assimilation of NH_4^+ or NO_2^-/NO_3^- by plants and microbes into their cell matrix and its conversion into organic nitrogen. Denitrification is the microbial reduction of nitrogen oxides (NO_3^- or NO_2^-) to N_2O or N_2. Nitrification is the oxidation of NH_4^+ to NO_3^-. DNRA is the reduction of NO_3^- to NH_4^+. Dinitrogen fixation is the reduction of N_2 to NH_3. Ammonia volatilization is the conversion of NH_4^+ to NH_3 within the soil/water environment resulting from a shift in pH (>7.5) (Fig. 1) (Myrold 1998; Mitsch and Gosselink 2000; Reddy and Delaune 2008).

The transformation processes listed above are largely chemically or biologically mediated. However, geological or physical processes can also retain nitrogen within a wetland ecosystem, such as cation/anion exchange where NH_4^+ or NO_3^- is bound within the sediment matrix by charged particles of clay or organic matter, thus immobilizing the NH_4^+ or NO_3^- molecule (Reddy and Delaune 2008). Nitrogen burial is also common in coastal environments, such as deltaic systems, where sediment accretion rates are high (Delaune et al. 1981; Delaune and Patrick 1990). This sedimentation process has been shown to sequester about 60% of inorganic nitrogen entering a Louisiana estuary (Smith et al. 1985). Due to the availability of certain inorganic and organic compounds, the various nitrogen transformation processes and flora-fauna competition, nitrogen is often considered the most limiting nutrient controlling the productivity of terrestrial, wetland and aquatic ecosystems (Vitousek et al. 1997; Mitsch and Gosselink 2000; Reddy and Delaune 2008).

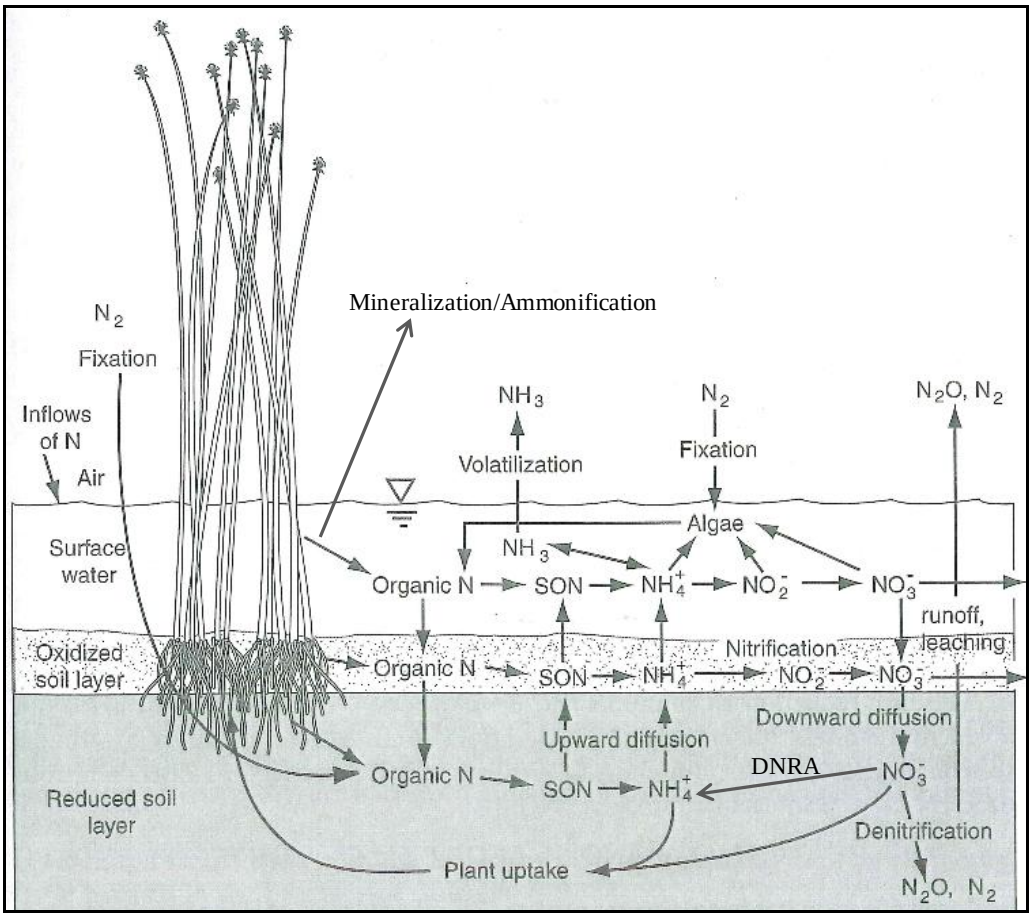

Fig. 1 Nitrogen transformations and common molecular forms in wetland ecosystems. SON indicates soluble organic nitrogen (ex. urea). DNRA indicates dissimilatory nitrate reduction to ammonium. Mineralization/ammonification process is shown in surface water, however, it is understood the process is also found to occur in the oxidized soil and reduced soil layer (after Mitsch and Gosselink, 2000).

Denitrification

Denitrification is the microbial process by which oxidized nitrogen compounds, NO_3^- and NO_2^-, serve as terminal electron acceptors during denitrifier respiration and are reduced to mainly inert N_2O and N_2 gases under anoxic conditions (Tiedje et al. 1989). During denitrification NO_3^-, oxidation state +5, is reduced to N_2, oxidation state 0, providing a transfer of five total electrons (e^-) used to generate cellular energy. The theoretical energy yield ($\Delta G°$) from the denitrification process is about 560 kJ mol^{-1} NO_3^- (Myrold 1998). The denitrification

process is predominantly carried out by facultative heterotrophic bacteria which obtain their energy through nitrogen oxide reduction coupled with low-molecular-weight organic carbon oxidation (Canfield et al. 2005). However, some denitrifiers are facultative chemolithoautotrophic bacteria which couple NO_3^- reduction with oxidation of inorganic species, such as ferrous (Fe^{2+}) iron, manganous (Mn^{4+}) manganese, or molecular hydrogen (H_2) oxidation (Canfield et al. 2005). When denitrification is coupled with electron transport phosphorylation it yields 60% of the energy (ATP) obtained through aerobic oxygen respiration, and is still considerably greater than the alternative fermentation pathway (Koike and Hattori 1975; Tiedje et al. 1989). Denitrifying bacteria represent approximately 0.1-5% of the total bacterial populations found in soils and sediments. Soil denitrifying bacteria are dominated by a few species from the genera *Pseudomonas*. A few species from the genera *Alcaligenes, Flavobacterium, and Bacillus* are also common denitrifiers (Tiedje 1988; Myrold 1998).

Denitrification is regulated by various abiotic and biotic factors. Denitrifier community composition and diversity is structured over the long-term by various conditions including: carbon substrate availability, temperature, moisture, oxygen (O_2) availability, pH, predation and large scale disturbances (i.e. fire, freeze/thaw, wetting/drying, etc). Denitrification activity at any particular moment is regulated by various environmental conditions and resources which influence the denitrifier community. The main environmental conditions and resources which regulate denitrification rates and kinetics are: soil O_2 content, electron acceptor (NO_3^-) availability, electron donor presence (i.e. organic carbon, reduced sulfur, H_2, Fe^{2+} and Mn^{4+}), plant root density, denitrification enzyme activity, temperature, pH, water residence time, sediment porosity and NH_4^+ and NO_3^- sediment-water exchange. Temperature is one important environmental factor which is known to regulate the microbial denitrifying community and

denitrification activity. Generally, a very broad temperature range (15-75 °C) has been observed for the denitrification process (Focht and Verstraete 1977). Denitrification activity generally increases exponentially with increasing temperature; however, this is usually limited to within the temperature range of the different enzymes, specific to denitrifiers, used in the denitrification process (Firestone 1982). Denitrifiers are thought to be ubiquitous in the soil environment which suggests denitrification rates are not limited by the denitrifier community itself, but instead by environmental conditions and resources (Wijler and Delwiche 1954; Firestone 1982; Twilley and Kemp 1986; King and Nedwell 1987; Reddy et al. 1989; Kemp et al. 1990; Mosier and Schimel 1993; Rivera-Monroy and Twilley 1996; Canfield et al. 2005; Wallenstein et al. 2006; Bianchi 2007; Reddy and Delaune 2008).

There are two main reactions in the denitrification process which depend on the NO_3^- source: 1) direct denitrification occurs when overlying water NO_3^- diffuses into the sediment anaerobic zone and is reduced (denitrified) to N_2 gas; 2) coupled nitrification-denitrification is fueled by NH_4^+ which is oxidized (nitrification) to NO_3^- within the water column or aerobic zone of sediment, and NO_3^- then diffuses into the anaerobic zone and is denitrified to N_2 gas (Jenkins and Kemp 1984; Cornwell et al. 1999). Direct denitrification is controlled by NO_3^- diffusion from the overlying water column into underlying sediments, and is established by a NO_3^- concentration gradient between the water-sediment interface (King and Nedwell 1987). This NO_3^- concentration gradient is controlled by: sediment porosity and the length of the diffusion path through the oxic sediment layer (Christensen et al. 1990). Coupled nitrification-denitrification is dependent on the following: sediment NH_4^+ and O_2 concentrations, N-mineralization rates, and the depth of O_2 penetration into the sediment (Nielsen 1992; Rysgaard et al. 1995). Whether direct and/or the coupled denitrification process is occurring both are

responsible for transforming reactive inorganic nitrogen into non-reactive N_2 gas, thus removing available nitrogen from the system.

Nitrate consumption is not limited to microbial denitrification alone. Other research suggests various NO_3^- removal processes exist in freshwater and marine systems such as, iron oxidation coupled to nitrate reduction (Hauck et al. 2001; Davidson et al. 2003; Weber et al. 2006), sulfide oxidation coupled to denitrification (Dannenberg et al. 1992; Fossing et al. 1995; Otte et al. 1999), dissimilatory nitrate reduction to ammonium (DNRA) (Tiedje 1988), and anaerobic ammonium oxidation "annamox" (Mulder et al. 1995; Thamdrup and Dalsgaard 2002; Dalsgaard et al. 2005). Denitrification and these other nitrate consumption pathways influence NO_3^- biogeochemistry and control the availability of NO_3^- for various ecological processes.

Denitrification: Isotope Pairing Technique Methodology

Denitrification is thought to remove a substantial NO_3^- fraction loaded to lakes, rivers, wetlands, continental shelf, deep sea and coastal estuarine sediments (Seitzinger 1988; Seitzinger et al. 1993; Cornwell et al. 1999). However, direct denitrification estimates often account for only half the total NO_3^- disappearance (Seitzinger 1988). NO_3^- disappearance has been assumed to be largely due to denitrification in Breton Sound, Louisiana, as NO_3^- enriched water comes in contact with benthic lake and marsh sediments (Lane et al. 1999; Bond 2006). Similarly, previous studies applying indirect (such as the acetylene reduction technique) denitrification measures in Breton Sound have supported the dominance of this pathway (Delaune and Jugsujinda 2003). This suggests NO_3^- removal via direct denitrification could be a prominent pathway in Breton Sound; however, the technique applied, and the mode of application to quantify denitrification does have its inherent limitations.

Different methods, both indirect and direct, have been developed for estimating denitrification including the following: the ^{15}N tracer method (Hauck and Melsted 1956), ^{15}N nitrate dilution method (Koike and Hattori 1978), direct measurement of N_2 gas production (Seitzinger et al. 1980), acetylene blockage (or inhibition) technique (Sorensen 1978), measurements of NO_3^- pore water profiles (Bender et al. 1977), N_2 flux using N_2:Ar ratio measured by membrane inlet mass spectrometer (MIMS) (Kana et al. 1994) and ^{15}N isotope pairing technique (Nielsen 1992). All these methods have their advantages and disadvantages. For example, the acetylene blockage technique has been widely applied due to its simplicity and rapid implementation; however, it is performed on sediment slurries, is known to underestimate denitrification by inhibiting nitrification, and thus reflects only the direct denitrification process (Seitzinger et al. 1993; Steingruber et al. 2001; Groffman et al. 2006). Conversely, direct measurement of N_2 gas production has its advantages in measuring both direct denitrification and coupled nitrification-denitrification, but becomes problematic due to the preservation of anoxic conditions, and as a result of the small N_2 production relative to the already high N_2 background (Seitzinger et al. 1980). The consequence of applying these methods is a dramatic shift from in-situ sediment structure and benthic processes to artificial environments which provide unrealistic denitrification estimates (Eyre and Ferguson 2007). As a result, more robust denitrification methods are desired; methods which maintain in-situ sediment structure and benthic processes, and also quantify direct and coupled denitrification, thus providing more realistic denitrification estimates.

The application of ^{15}N-labeled NO_3^- to terrestrial soil and measuring the accumulated ^{15}N-labeled gas products over time was the first method developed for estimating denitrification (Hauck and Melsted 1956). However, identifying the source which contributes to these ^{15}N-

labeled gas products became complicated because several sources could potentially contribute ^{14}N and ^{15}N isotopes (Hauck and Melsted 1956). This problem was addressed by demonstrating how the ratio of the two N_2 products, $^{14}N^{15}N$ ($^{29}N_2$) and $^{15}N^{15}N$ ($^{30}N_2$), can reflect the ratio in the ^{14}N and ^{15}N isotopes from the source, assuming the N_2 product formed is by random isotope pairing (Hauck et al. 1958). The original work by Hauck et al. (1958) continued on terrestrial soils until 1992 when it was first implemented in an aquatic study and became referred to as the ^{15}N isotope pairing technique (IPT) (Nielsen 1992).

The IPT method relies on the natural abundance of stable nitrogen isotopes, 99.64% ^{14}N and 0.36% ^{15}N (Nielsen 1992). In IPT experiments a known $^{15}NO_3^-$ concentration (minimum 98% ^{15}N) is added to the water column in an experimental system where $^{15}NO_3^-$ can diffuse into the sediment over time and be directly denitrified, or $^{15}NO_3^-$ can mix with ambient $^{14}NO_3^-$ molecules found within the sediment interstitial water and be denitrified. A result from these two pathways is denitrification which can produce N_2 molecules with atomic molecular masses $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$ (Steingruber et al. 2001). In ambient atmosphere, the $^{29}N_2$ and $^{30}N_2$ atomic masses only constitute 0.7299 and 0.001%, respectively, of the entire atmospheric N_2 pool (Nielsen 1992).

The IPT has its advantage over many other techniques by quantifying both direct denitrification and coupled nitrification-denitrification from labeled $^{15}NO_3^-$ added to the overlying water of an experimental system (Steingruber et al. 2001). The IPT can be performed on intact sediment cores, which preserves sediment structure and benthic processes. It also provides reliable estimates without "over fertilizing" the experimental system by using $^{15}NO_3^-$ molecules with a minimum of 98% ^{15}N atoms. This high ^{15}N atom abundance in the $^{15}NO_3^-$ molecule allows for incubation concentrations to be used which reflect ambient concentrations

found within the ecosystem of study, but also assures easy quantification of labelled ^{15}N over background ^{15}N levels. Thus, by applying labelled $^{15}NO_3^-$ concentrations, which reflect ambient conditions, the IPT can provide near in-situ denitrification estimates.

The IPT is based on four main assumptions, which have the potential to limit the techniques implementation and interfere with the interpretation of denitrification rates (Nielsen 1992; Rysgaard et al. 1995; Eyre et al. 2002). However, multiple tests can be performed to insure these assumptions are appropriately applied to denitrification estimates.

The first assumption requires a stable NO_3^- concentration gradient become established across the sediment-water interface shortly after $^{15}NO_3^-$ addition. The conceptual model (Fig. 2) suggests too fulfill this assumption the production of $^{29}N_2$ and $^{30}N_2$ relies on ^{15}N atoms from the $^{15}NO_3^-$ in the overlying water. Therefore, if a stable $^{15}NO_3^-$ gradient is established then the production of $^{29}N_2$ and $^{30}N_2$ should increase linearly with time over the experiment duration. This linearity indicates $^{15}NO_3^-$ was immediately available to the denitrifying community, and the assumption was fulfilled (Nielsen 1992; Rysgaard et al. 1995; Eyre et al. 2002).

The second assumption requires direct denitrification from $^{15}NO_3^-$ to increase linearly as $^{15}NO_3^-$ concentrations increase in the overlying water column. This assumption is evaluated by adding different $^{15}NO_3^-$ concentrations to the overlying water and determining the direct denitrification rate. The conceptual model (Fig. 2) identifies that to fulfill this assumption as $^{15}NO_3^-$ concentrations increase in the water column, direct denitrification should increase linearly. However, at elevated $^{15}NO_3^-$ concentrations direct denitrification may no longer maintain a linear relationship due to Michaelis-Menton type saturation effects (Steingruber et al. 2001). If fulfilled this second assumption suggests direct denitrification was increasing as the

overlying water $^{15}NO_3^-$ concentration increased (Nielsen 1992; Rysgaard et al. 1995; Eyre et al. 2002).

The third assumption requires the added $^{15}NO_3^-$ will not interfere with the coupled denitrification rate based on $^{14}NO_3^-$ and that homogenous isotope mixing is occurring. This is a two part assumption, and using the conceptual model (Fig. 2) suggests if increasing $^{15}NO_3^-$ concentrations do not interfere with the coupled denitrification rate based on $^{14}NO_3^-$ than coupled denitrification estimates should remain independent of increasing $^{15}NO_3^-$ concentration additions to the overlying water column. The second part requires the added $^{15}NO_3^-$ mix homogenously with the $^{14}NO_3^-$ pool in the sediment. Sediment heterogeneity, bioturbation, inhomogeneous nitrification activity and other phenomena can lead to deviations in the mixing of the two isotopes. Considering the conceptual model (Fig. 2) the production of $^{29}N_2$ requires a ^{15}N atom pair with a ^{14}N atom from within the sediment, and if the two isotopes are not homogenously mixed, then this pairing will be irregular, which will result in $^{29}N_2$ production being low relative to $^{30}N_2$ production. This will lead to an underestimate in coupled denitrification activity. Therefore, coupled denitrification activity should remain independent of increasing $^{15}NO_3^-$ concentration additions to fulfill the second part of the third assumption. If fulfilled this third assumption indicates no interference of $^{15}NO_3^-$ additions on coupled denitrification and homogenous isotope mixing was occurring in the sediment (Nielsen 1992; Rysgaard et al. 1995; Eyre et al. 2002).

Finally, assumption four deals with the anaerobic ammonium oxidation (anammox) reaction. Annamox is a microbial process performed by chemolithoautotrophic bacteria where NH_4^+ is oxidized under anoxic conditions with NO_2^- or NO_3^- acting as the terminal electron acceptor. Annamox, like denitrification, does result in N_2 gas formation as the end product from

the reaction (Mulder et al. 1995; Van De Graaf et al. 1995). Specific experiments need to be conducted to evaluate the N_2 proportion produced via the annamox reaction, otherwise coupled nitrification-denitrification may be overestimated (Rysgaard et al. 1995; Lohse et al. 1996; Steingruber et al. 2001; Eyre et al. 2002).

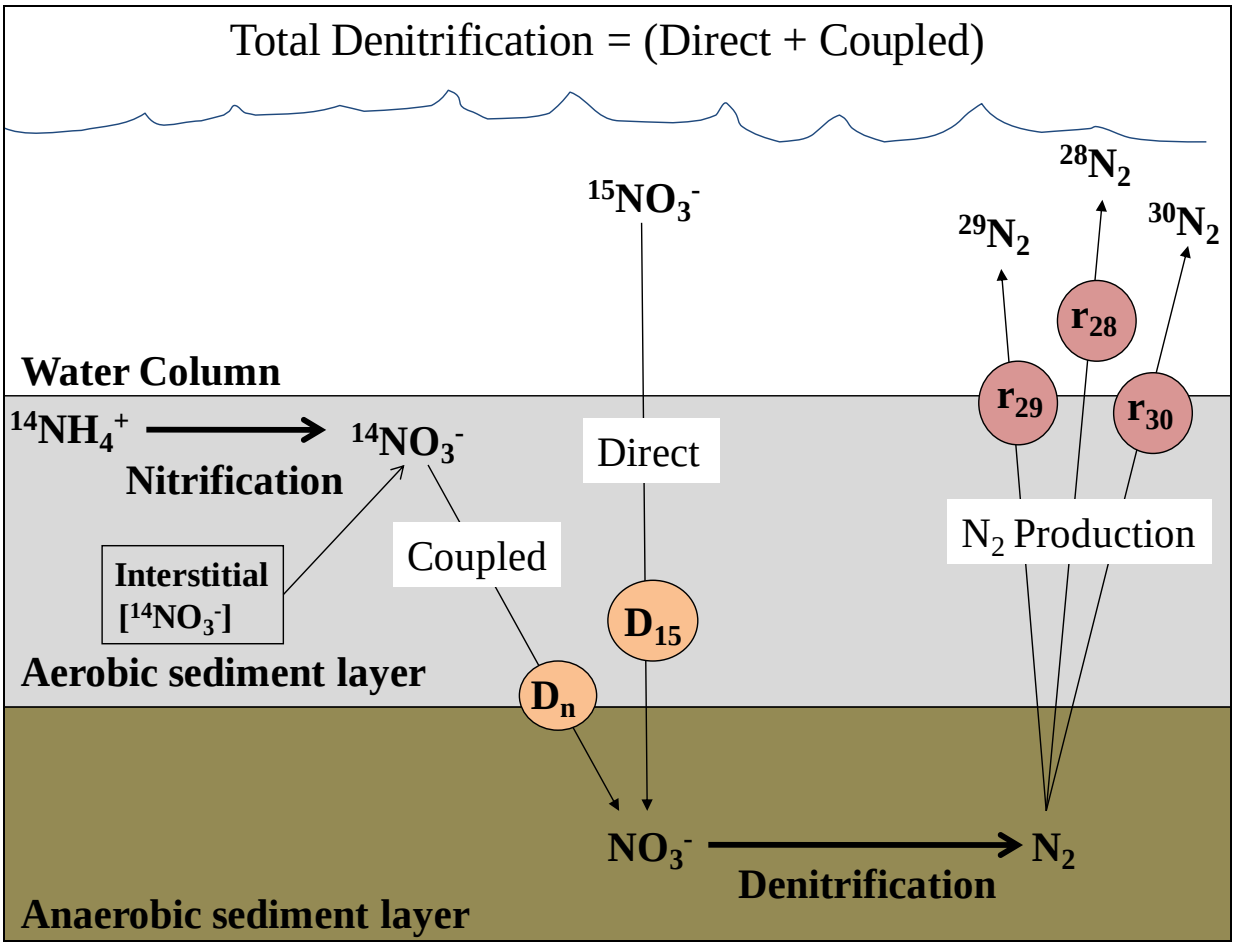

Fig. 2 Isotope pairing technique conceptual model applied during current study. Diagram illustrates contribution of ^{15}N and ^{14}N atoms via direct and coupled denitrification to labeled $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$ products (after Steingruber et al. 2001).

When the four assumptions above are fulfilled, the denitrification rate can be calculated by following the applied stoichiometry from the denitrification process; $^{14}NO_3^-$ and $^{15}NO_3^-$ reduction forms the following isotope pairs $^{14}N^{14}N$ ($^{28}N_2$), $^{14}N^{15}N$ ($^{29}N_2$) and $^{15}N^{15}N$ ($^{30}N_2$),

which have known distributions within the atmospheric N_2 pool (Lohse et al. 1996). As a result, the dissolved $^{28}N_2$ concentration is determined using an empirical formula which incorporates temperature, salinity and multiple N_2 solubility coefficients (Weiss 1970; Hamme and Emerson 2004). The $^{29}N_2$ and $^{30}N_2$ (M) concentrations from the incubated sediment cores are calculated using the $^{28}N_2$ concentration as follows (shown here for $[^{29}N_2]$):

$$[^{29}N_2] = \frac{[^{29}N_2]}{[^{28}N_2]} \cdot [^{28}N_2]; \quad (1)$$

where the brackets indicate the N_2 concentration and $[^{29}N_2] / [^{28}N_2]$ is the ratio obtained from the incubated sediment core gas sample determined with a isotope ratio mass spectrometer (Nielsen 1992).

Isotope pair production, $^{29}N_2$ (r_{29}) and $^{30}N_2$ (r_{30}) (mol N_2 m^{-2} hr^{-1}), is obtained from the $^{29}N_2$ or $^{30}N_2$ concentration calculated above (shown here for r_{29}):

$$r_{29} = \frac{m_{29}}{A} \cdot (V_w + \phi V_s); \quad (2)$$

where m_{29} is the slope of the linear regression line from $[^{29}N_2]$ plotted against time, A is the incubated sediment surface area, V_w is the incubated water volume, Φ is the sediment porosity and V_s is the incubated sediment volume.

Denitrification rates from $^{15}NO_3^-$ and $^{14}NO_3^-$ can be estimated using the production rates (r_{29} and r_{30}) as follows:

$$D_{15} = r_{29} + 2 \cdot r_{30}; \quad (3)$$

$$D_{14} = D_{15} \cdot \frac{r_{29}}{2 \cdot r_{30}}; \quad (4)$$

where D_{15} is denitrification from $^{15}NO_3^-$ and D_{14} is denitrification from $^{14}NO_3^-$.

$$D^{tot} = D_{14} + D_{15} \; ; \tag{5}$$

$$D_n = D^{tot} - D_W^{tot} \; ; \tag{6}$$

$$D_w{}^{tot} = \frac{D_{15}}{\varepsilon} \; ; \tag{7}$$

$$D_W = D_W^{tot} \cdot (1 - \varepsilon) \; ; \tag{8}$$

where D_{tot} is the total denitrification in the sediment, D_n is coupled nitrification-denitrification, $D_w{}^{tot}$ is the denitrification from the $^{14}N/^{15}N$ mixture diffusing from the overlying water, ε is the isotopic nitrate enrichment during the incubation and D_w is the natural direct denitrification rate (Nielsen 1992; Steingruber et al. 2001). Under the circumstance there is no initial $^{14}NO_3^-$ concentration in the water the added $^{15}NO_3^-$ is not diluted, and therefore $D_w{}^{tot}$ corresponds to direct denitrification (D_{15}), D_w is zero, and coupled nitrification-denitrification (D_n) is equal to D_{14} (Nielsen 1992; Steingruber et al. 2001) A step by step example showing how denitrification rates are calculated from an experiment in March 2008 is presented (Appendix E).

To apply the IPT and measure denitrification in the current study, experimental glass cores were designed to be 12 cm long with a 2 cm internal diameter and a total volume of 41.6 cm^3. Once sediment was collected the core contained ca. 9 to 10 cm overlying water and ca. 2 to 3 cm sediment (Fig. 3A). A water bath incubation system was developed for use during denitrification experiments (Fig. 3B). The water bath incubation system oscillates at ca. 30 rpm, and was designed to minimize disturbance across the sediment-water interface, provide constant

water temperature during experiments and aid in the $^{15}NO_3^-$ diffusion from the overlying water into the sediment.

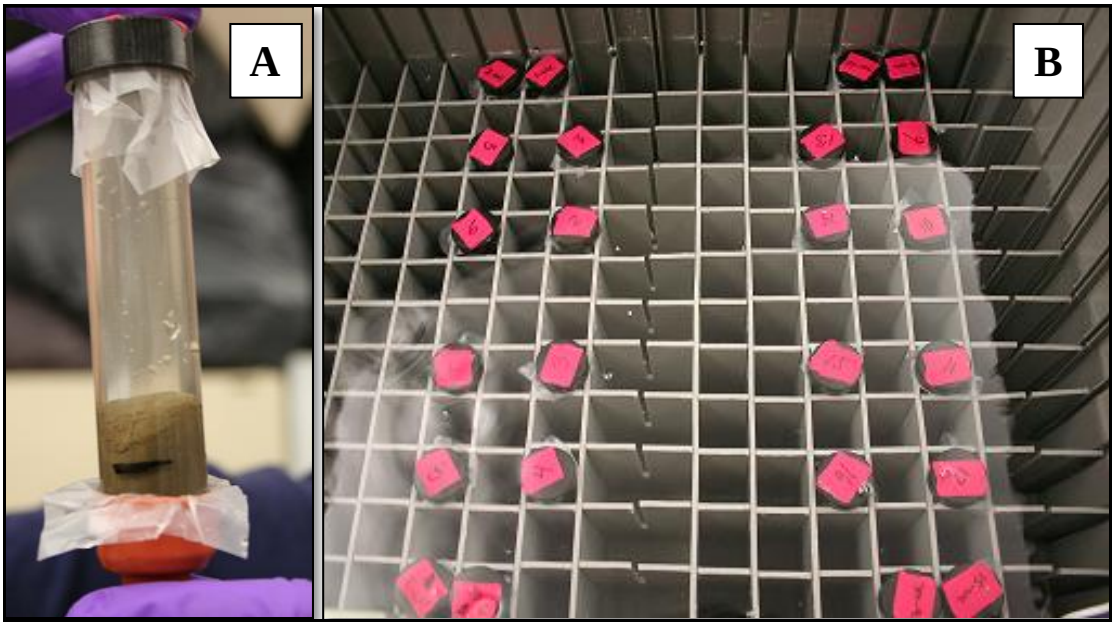

Fig. 3 Sediment-water experimental core used to measure denitrification activity in upper Breton Sound (A). Water bath incubation system used to oscillate sediment-water experimental cores (B).

To analyze collected headspace gas samples from IPT experiments for the $^{14}N^{15}N$ ($^{29}N_2$) and $^{15}N^{15}N$ ($^{30}N_2$) abundance and concentration a gas chromatograph coupled to a triple-collector 20/20 isotope ratio mass spectrometer was utilized with standard protocols (GC-MS, RoboPrep-G plus, Europa Scientific, SerCon Ltd, UK). Although other techniques for ^{15}N analysis have been employed, the isotope ratio mass spectrometry is the preferred method because its precision is unmatched. The instrument contains three major components: the analyzer tube, the ion source, and the detectors. The analyzer tube is curved at ca. 90° angle with the ion source and detector mounted at opposite ends. A N_2 gas sample enters the system via an inlet port where it is initially passed through a column containing anhydrous magnesium perchlorate (to remove water vapor) and Carbosorb (to trap carbon dioxide (CO_2)). The N_2 gas sample then flows into a

gas chromatograph column where components of interest are separated and leaked into the ion source on the mass specrometer. Once in the ion source the N_2 is bombarded with electrons by a heated filament, which results in electron beam acceleration across the inflowing N_2 sample. This creates a positive potential between the heated filament and an anode trap, such that the energy from the electrons is much greater than the ionization potential of N_2. This result causes N_2 to ionize, which is a loss in electrons, forming $^{28}N_2^+$, $^{29}N_2^+$ and $^{30}N_2^+$. These positive ions are drawn out from the ion source by the combined effects from a small positive potential, created by the repeller, and a strong negative potential created across the accelerating electrodes. The ions continue into the analyzer tube where an electromagnetic field curves the ions path, with the degree of curvature relative to the ion's mass, charge and velocity. The charge and velocity is held constant by the accelerating voltage, created from the accelerating electrodes, thus causing the ions to separate into three beams by their mass-to-charge ratio. The ion's then reach the detector in the following order $^{28}N_2^+$, $^{29}N_2^+$ and $^{30}N_2^+$. Once separated they collide with the detector where ion beams are amplified, producing a proportional voltage which is measured on a chart recorder (Mulvaney 1993).

Denitrification Rates from the Mississippi River Delta

Agricultural and anthropogenic activities throughout the Mississippi River drainage basin supply an unprecedented annual nitrogen flux to the Gulf of Mexico of 1.56×10^6 Mg N, of which 62% is NO_3^- (Goolsby and Battaglin 2001). This nitrogen flux accounts for 90% of the total nitrogen discharged annually to the Gulf of Mexico (Dunn 1996), and this been correlated with the largest (up to 21,000 km^2) anthropogenic bottom water hypoxic zone in the western hemisphere (Alexander et al. 2000; Goolsby and Battaglin 2001; Turner et al. 2005; Turner et al.

2008). Due to this large nitrogen flux entering into coastal Louisiana ecosystems, denitrification research has been prevalent in Louisiana; approximately 32 different published studies have quantified denitrification over the last 28 years across the coastal Louisiana wetland landscape (Fig. 4).

A literature review of published studies evaluating denitrification in Louisiana was compiled (Appendices A-C) (Rivera-Monroy et al. 2009, in review). These studies from Louisiana employed a range of techniques, both indirect and direct, to estimate denitrification rates. Denitrification rates from these associated studies were grouped into three categories during the literature review: 1) ^{15}N techniques, 2) acetylene inhibition technique and 3) indirect techniques (Appendices A-C). Mean denitrification rates from the IPT in my study (presented in Chapter 2) are included in this review for comparison (Appendix A). The ^{15}N techniques applied in the denitrification studies are: $^{15}N_2$ emission, ^{15}N dilution, ^{15}N static chamber and ^{15}N recovery. The indirect techniques applied were: N_2O emission, NO_3^- disappearance, mass balance and stoichiometry. Denitrification investigations were performed on sediments from different ecosystems including: marshes, estuarine and lake benthic environments, forested wetlands and the continental shelf with most studies in areas hydrologically influenced by either the Atchafalaya or Mississippi Rivers. Louisiana west of Vermillion Bay, commonly referred to as the Chenier plain, had no published denitrification research found during the literature review.

All denitrification rates in the review were converted to μmol N m^{-2} h^{-1} to provide easy comparison and all N_2O measurements were converted to N_2 using N_2:N_2O ratios provided by the author or from other published literature sources. Some published denitrification studies were not included in the summary because the denitrification rates were presented on a soil weight basis making conversion to an area basis impractical. Denitrification rates ranged from

2.7-2852.8 µmol N m^{-2} h^{-1} for the ^{15}N isotope techniques (Appendix A), 0.2-1338.6 µmol N m^{-2} h^{-1} for the acetylene inhibition technique (Appendix B) and 21-2157.7 µmol N m^{-2} h^{-1} for the indirect techniques (Appendix C).

Considering all studies from Louisiana 82% employed the acetylene inhibition technique and the ^{15}N isotope methods. The acetylene inhibition technique and ^{15}N isotope methods each equally accounted for 41% of the total studies. The indirect methods accounted for 18% of the total studies. All denitrification studies had various experiment conditions (i.e. sediment slurries vs. intact cores, high NO_3^- concentrations relative to background NO_3^- levels, long vs. short experiment duration, field vs. lab, etc) making comparison among techniques and between ecosystem types futile. To address this it is recommended that future investigations try to incorporate multiple techniques to measure denitrification, while maintaining similar experimental conditions, with the goal to reduce the variability in denitrification estimates previously obtained. This will enhance our local understanding for how prevalent denitrification is in coastal Louisiana.

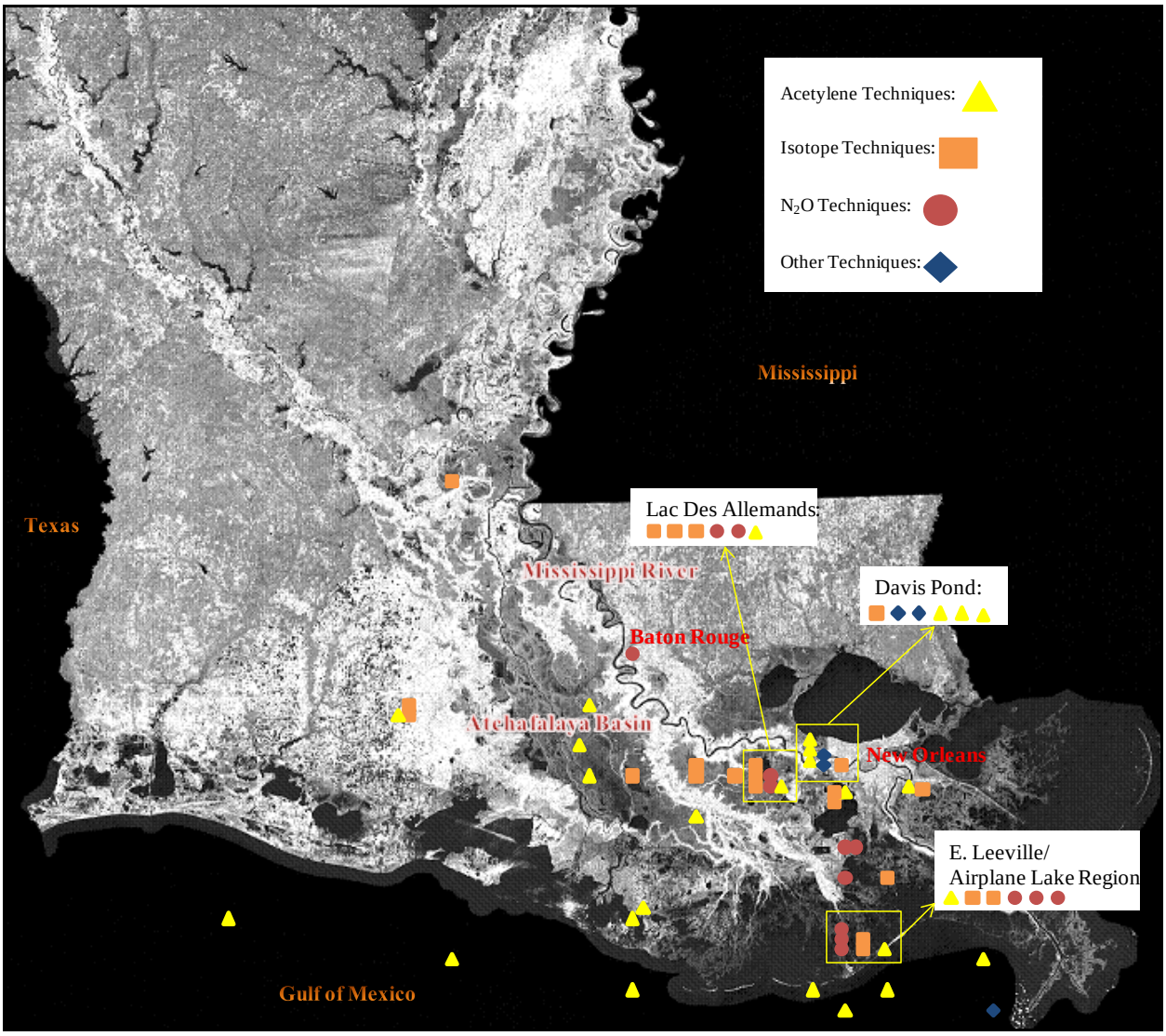

Fig. 4 A map of Louisiana illustrating the spatial extent and diversity of denitrification techniques applied to estimate denitrification rates throughout the period 1981-2009 (Modified from Rivera-Monroy et al. 2009, in review).

CHAPTER 2. COMPARING DENITRIFICATION ESTIMATES ACROSS DIFFERENT HABITAT TYPES AND WATER TEMPERATURES IN BRETON SOUND, LOUISIANA

Introduction

Human activity over the last century has greatly altered the global nitrogen cycle through synthetic nitrogen fertilizer creation and their application on the terrestrial landscape (Vitousek et al. 1997; Howarth et al. 2000; Vitousek et al. 2002). As a result, nitrate (NO_3^-) concentrations have increased in watershed surface and subsurface waters (Firestone 1982; Carpenter et al. 1998) which are eventually transported to many coastal water bodies. This can enhance the potential for eutrophication and create economic, environmental and social problems (Nixon et al. 1996; Howarth et al. 2000; Galloway et al. 2002; Howarth et al. 2002; Vitousek et al. 2002). More than 60 percent of coastal waters, and the rivers which empty into them, in the continental United States are impaired (i.e. not suitable for use in drinking, irrigation, industry, recreation or fishing) from nutrient pollution (Carpenter et al. 1998; Howarth et al. 2000). For example, the NO_3^- flux from the Mississippi River today is two to three times greater than in 1955 (Vitousek et al. 1997; Goolsby and Battaglin 2001), and this flux has been correlated with the largest zone (up to 21,000 km^2) of anthropogenic bottom water hypoxia in the western hemisphere (Turner et al. 2005; Turner et al. 2008). Due to the excess inorganic nitrogen in many coastal areas, including coastal Louisiana, there is particular interest in understanding and implementing effective nutrient management strategies. One such nutrient reduction strategy is denitrification, the conversion of NO_3^- to nitrogen (N_2O, N_2) gas. Quantifying environmental controls on denitrification in aquatic systems, especially in coastal Louisiana benthic subtidal and marsh habitats, is essential for implementing future nutrient management approaches.

Over the years, Louisiana has implemented Mississippi River freshwater diversions along the lower river sections with the goal to lower salinities in coastal wetlands (USACOE 2006). The Old River control structure, 87 km upriver of Baton Rouge, near Simmesport, LA regulates approximately 30% of the combined Mississippi and Red River flow diverted into its present major distributary, the Atchafalaya River (Johnson et al. 1985). South of Baton Rouge there are three major freshwater diversion structures in operation: 1) Bonnet Carré spillway, 2) Davis Pond and 3) Caernarvon. The Bonnet Carré is the largest (708 $m^3 s^{-1}$ maximum discharge), positioned 33 miles upstream of New Orleans, Louisiana, and has been discharging into Lake Pontchartrain since 1937. Bonnet Carré is only opened when New Orleans is susceptible to flooding from the Mississippi River, and since 1937 it has only been opened nine times (1937,1945, 1950, '73, '75, '79, '83, '97 and 2008) (USACOE 2004). The second largest is Davis Pond (302 $m^3 s^{-1}$ maximum discharge), situated 22 miles upstream of New Orleans and has been flowing into Barataria basin since 2002. The smallest of the three is Caernarvon (226 $m^3 s^{-1}$ maximum discharge), located 15 miles downstream of New Orleans and has been impacting Breton Sound basin since 1991 (USACOE 2006). Combined, the Atchafalaya and Mississippi Rivers contribute about 80% of the freshwater discharged from the United States to the Gulf of Mexico (Dunn 1996). The two rivers also contribute 90% of the total nitrogen to the Gulf of Mexico (Dunn 1996) with approximately 62% of the total nitrogen as NO_3^- (Goolsby and Battaglin 2001).

Breton Sound, a wetland-estuarine basin created several thousand years ago by the former Plaquemines-St. Bernard delta complex (Scruton 1960; Roberts 1997), and isolated from the Mississippi River prior to and following the 1927 great flood, has partially reclaimed its hydrologic character via the Caernarvon freshwater diversion structure. The Caernarvon

freshwater diversion seasonally influences approximately 260 km^2 of benthic and marsh habitats in upper Breton Sound (Mitsch et al. 2005). The original authorization for operation was to re-establish salinity conditions in Breton Sound (USACOE 2006). However, Caernarvon has been operated in the past (ex. 2001-2003) to provide freshwater pulsing events, which mimic historic flooding events, by discharging large amounts (> 100 $m^3 s^{-1}$) of Mississippi River water during late winter and spring under high Mississippi River stages. Unfortunately, unlike historic freshwater pulses, the pulses today provide an unprecedented inorganic nitrogen pulse, as NO_3^- (annual average 71.4 µM NO_3^-), which raises concerns about degrading water quality conditions in the Breton Sound estuarine system.

Mississippi River water temperature in the late winter and early spring is < 8 °C due to snow melt within the upper region of the Mississippi River basin. Consequently, as the water travels downstream through the United States and into Louisiana it maintains this cold water temperature (about 6 °C) (Lane et al. 2007). As a result, water temperature is low when Mississippi River water is diverted into Breton Sound during late winter and spring. However, Mississippi River water which floods marshes of the upper basin during a freshwater pulse event can increase in water temperature from < 8 °C to > 22 °C, due to solar radiation which gradually heats the shallow overland marsh flow. The magnitude of water temperature increase as water flows over the marsh surface depends on: Caernarvon diversion discharge rate, season, local estuarine water temperature and local meteorological conditions (Robert Twilley, personal communication).

At low Caernarvon discharge rates (< 125 $m^3 s^{-1}$) diverted waters mainly travel downestuary through the Breton Sound basin as channelized flow (e.g. channels, bayous and lakes). However, when Caernarvon discharge rates exceed 125 $m^3 s^{-1}$, the capacity for

channelized flow is exceeded and diverted river water overbanks the adjacent marsh surface initiating overland flow across the freshwater marsh (Snedden et al. 2007). In general, overland flow over the freshwater marsh surface occurs in a downestuary direction at velocities < 0.05 m s^{-1} (Snedden et al. 2007). Due to the low overland flow velocity, lower water residence time causes water temperatures to increase as diverted water travels downestuary over the marsh surface. Measurements in upper Breton Sound marsh habitats indicate as overland flow moves downestuary across the marsh surface solar radiation causes an increase in water temperature from < 8 °C to > 20 °C (Baker 2005; Bond 2006).

One important control on nitrogen biogeochemistry processes across the upper Breton Sound landscape appears to be water temperature. For example, surveys from nitrogen flux and flume experiments from upper Breton Sound freshwater marshes, during high discharge periods (>125 m^3 s^{-1}) when overland flow was initiated, indicates diverted Mississippi River water with low water temperatures (< 8 °C) limits NO_3^- uptake rates (Baker 2005; Bond 2006). Similar measurements in Breton Sound, during low discharge periods (< 125 m^3 s^{-1}) when channelized flow occurs, suggests less NO_3^- uptake when diverted Mississippi River water temperature is low (< 8 °C) (Lane et al. 1999; Lane et al. 2007).

However, as water travels downestuary in Breton Sound, whether via channelized flow or overland marsh flow, water residence time increases and water temperature has been shown to increase. A twenty-four hour investigation, which examined a 2.5 km transect over the marsh surface with the upstream sampling station located approximately 4 km downestuary from Caernarvon, measured low water temperatures (< 8 °C) and high NO_3^- concentrations (110 µM) at the upstream sampling station. As overland flow occurred over this 2.5 km distance the downstream sampling station, 6.5 km downestuary from Caernarvon, had increased water

temperatures (> 20 °C) and decreased NO_3^- concentrations (< 10 µM) (Robert Twilley, personal communication). Similarly, as Mississippi River water travels downestuary via channelized flow it enters large lakes, such as Big Mar and Lake Leary within the upper Breton Sound basin. This results in a decrease in water velocity and an increase in water residence time (Lane et al. 2007), which can result in an increase in water temperature and a decrease in NO_3^- concentrations as water flows downestuary through estuarine waterways (Lane et al. 1999). Research in Breton Sound and other coastal Louisiana basins suggest NO_3^- levels in diverted Mississippi River water are rapidly reduced as water temperature increases while river water propagates downestuary via estuarine waterways (Lane et al. 1999; Lane et al. 2002; Lane et al. 2004; Lane et al. 2007). However, what is not clear is whether the NO_3^- loss observed in these marsh and estuarine waterway habitats is primarily a result of denitrification or a combination of other processes, such as: dilution with ambient estuarine water, phytoplankton uptake, plant uptake, burial or reduction to NH_4^+. Similarly, it is not clear to what extent the denitrification process controls this transition of high nitrate with low water temperatures (< 8 °C) to low nitrate in downestuary stations that have warmer water temperatures (> 20 °C) in upper Breton Sound habitats.

As a result of the Caernarvon diversion structure, habitat and water temperature appear to be important factors influencing nitrogen biogeochemistry within upper Breton Sound. Consequently, different water temperatures (i.e. < 8 °C and > 20 °C) and different habitat types (i.e. marsh and benthic) can result in different denitrification capacities due to varying environmental conditions. A previous denitrification study in a benthic habitat (Big Mar Lake) located downstream from the Caernarvon diversion structure suggests Breton Sound benthic habitats have the potential capacity to remove significant NO_3^- quantities. Using an indirect method, the acetylene inhibition technique, potential denitrification rates ranged from 13.7 to

349.8 μmol N m^{-2} h^{-1} in Big Mar benthic sediments (Delaune and Jugsujinda 2003). However, extrapolating these potential denitrification estimates are limited due to the uncertainty associated with the acetylene method, and due to the uncertainty surrounding environmental factors such as water temperature and habitat type. The extent to which denitrification occurs in benthic and marsh habitat sediments, as well as over different water temperatures, is important information regarding the fate of NO_3^- from the Caernarvon diversion structure in the Breton Sound estuary. As a result, upper Breton Sound is analogous to a denitrification experimental coastal basin; high NO_3^- concentrations along with varying water temperatures and flow paths provide an optimal area to understand environmental controls (i.e. water temperature and different habitat types) on landscape patterns of denitrification.

Different methods, both indirect and direct, have been developed for estimating denitrification: the ^{15}N tracer method (Hauck and Melsted 1956), the ^{15}N-NO_3^- dilution method (Koike and Hattori 1978), direct measurement of N_2 gas production (Seitzinger et al. 1980), acetylene blockage (or inhibition) technique (Sorensen 1978), measurements of NO_3^- pore water profiles (Bender et al. 1977), N_2 flux using N_2:Ar ratio measured by membrane inlet mass spectrometer (MIMS) (Kana et al. 1994) and ^{15}N isotope pairing technique (IPT) (Nielsen 1992). All these methods have their advantages and disadvantages. For example, the acetylene inhibition technique has been widely applied due to its simplicity and rapid implementation; however, it is performed on sediment slurries, and is known to regularly underestimate denitrification by inhibiting nitrification (Seitzinger et al. 1993; Steingruber et al. 2001; Groffman et al. 2006). Conversely, direct N_2 gas production measurements have advantages in measuring both direct denitrification and coupled nitrification-denitrification, but the method becomes problematic due to anoxic condition preservation, and due to the small N_2 production

relative to the already high N_2 background (Seitzinger et al. 1980). The consequence of applying these methods is a dramatic shift from in-situ sediment structure and benthic processes to artificial environments which provide unrealistic in-situ denitrification estimates (Eyre and Ferguson 2007). Therefore, more robust denitrification methods are desired; methods which maintain in-situ sediment structure and benthic processes, quantify both direct and coupled denitrification, and use NO_3^- enrichment solutions which reflect ambient estuarine NO_3^- concentrations, thus providing more reliable denitrification estimates.

The IPT has its advantage over many other techniques by quantifying both direct denitrification and coupled nitrification-denitrification from only $^{15}NO_3^-$ additions. The IPT can be performed on intact sediment cores, which preserves sediment structure and benthic processes. It also provides reliable estimates without "over fertilizing" the experimental system by using $^{15}NO_3^-$ molecules with a minimum of 98% ^{15}N atoms. This high ^{15}N atom abundance in the $^{15}NO_3^-$ molecule allows for incubation concentrations to be used which reflect ambient concentrations found within the ecosystem of study, but also assures easy quantification of labelled ^{15}N over background ^{15}N levels. Thus, by applying labelled $^{15}NO_3^-$ concentrations, which reflect ambient conditions, the IPT can provide near in-situ denitrification estimates. The IPT is based on four main assumptions, which have the potential to limit the techniques implementation and interfere with the interpretation of denitrification rates (Nielsen 1992; Rysgaard et al. 1995; Eyre et al. 2002). However, multiple tests can be applied to insure these assumptions are appropriately applied to denitrification estimates.

In coastal Louisiana, an enormous effort has been devoted to measuring denitrification using many different techniques; with comparison among studies and across different habitats being futile due to varying experimental and methodological differences. Furthermore, no

published denitrification studies, to date, have applied the IPT in Louisiana. I report here the first IPT denitrification estimates from intact sediment cores in estuarine waterway (i.e. benthic habitats) and marsh habitats from upper Breton Sound, Louisiana. My first objective was to conduct laboratory experiments to fulfill the different IPT assumption requirements at four different locations in upper Breton Sound. The second objective was to evaluate the influence different water temperatures and habitat types have on denitrification rates. I addressed the following questions. First, were the IPT assumptions fulfilled at the four chosen locations? If so, what is the influence two different water temperatures (8 and 22 °C treatments) have on denitrification estimates? Third, how do different habitat types (marsh and benthic) influence denitrification estimates? Finally, how do denitrification estimates from my study compare with other IPT studies from around the world, as well as to denitrification and NO_3^- flux estimates obtained from upper Breton Sound.

Materials and Methods

Study Area

The study was carried out in upper Breton Sound estuary, located southeast of New Orleans along coastal Louisiana. The Breton Sound estuary (Fig. 5) is approximately 40 km in length, has an 1100 km^2 area and is connected to the Gulf of Mexico at its southern boundary (Lane et al. 1999; Swenson et al. 2006). The freshwater divide in Breton Sound occurs at about 20 km downstream from the Caernarvon diversion structure (Swenson et al. 2006). The estuary consists of a pro-delta clay foundation over which a landscape of fresh, brackish, and saline marshes, barrier islands, natural levees, and former Mississippi distributaries are positioned (Coleman et al. 1998). The estuary is hydrologically contained by the Mississippi River levee

system to the west, the Mississippi River Gulf Outlet spoil banks to the east, and the Bayou La Loutre natural levees to the north.

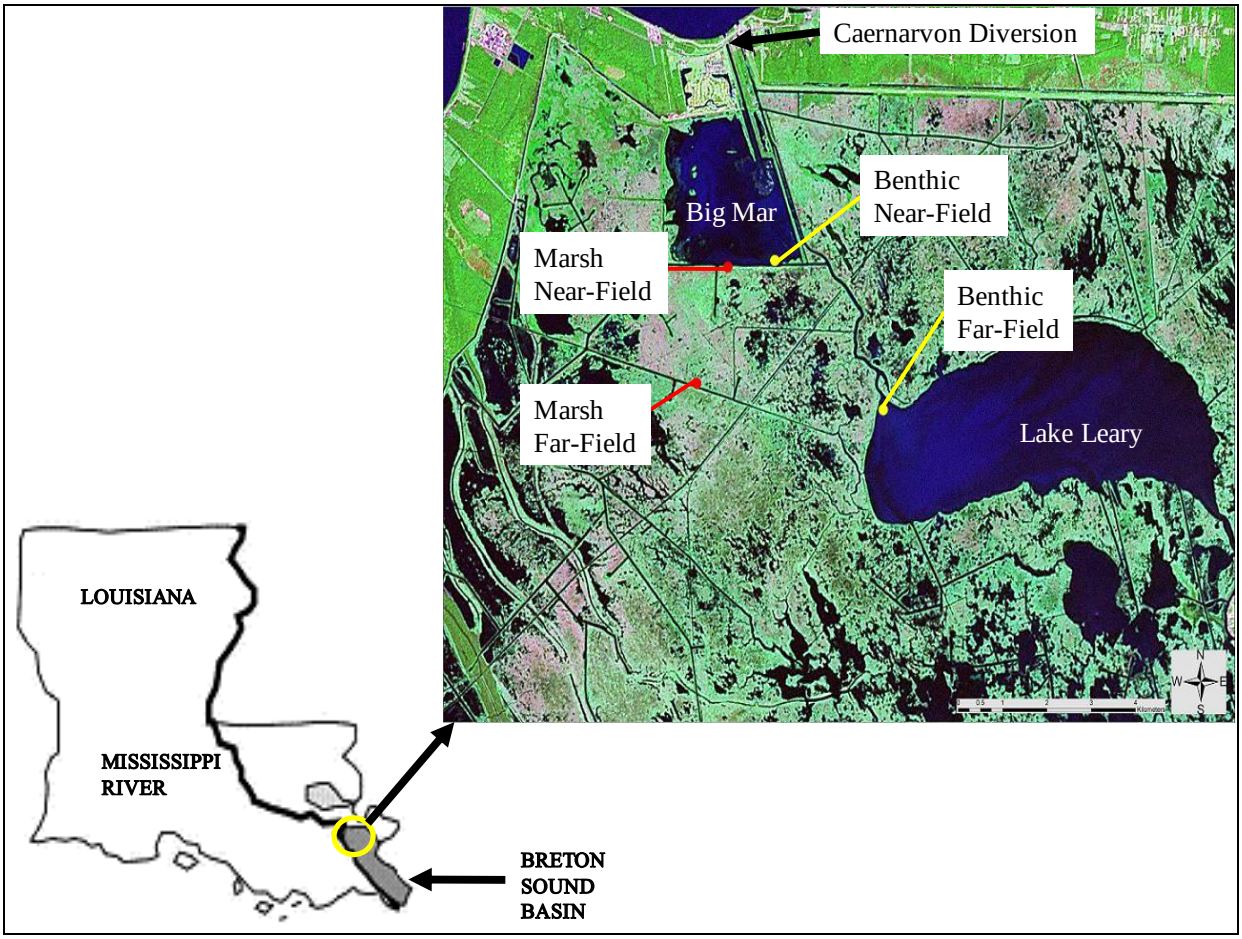

Fig. 5 Louisiana State showing the Mississippi River and Breton Sound basin location. Upper Breton Sound basin is circled in yellow and enlarged with the four sampling locations shown. (modified from Lane et al. 1999).

The upper Breton Sound estuary is predominantly colonized by *Spartina patens*, a brackish marsh plant species. However, due to salinity reductions, the Caernarvon diversion has promoted the establishment of more freshwater marsh and submersed aquatic species, such as: *Typha* spp. (Cattail), *Alternanthera philoxeroides* (Alligator weed), *Colocasia antiquorum* (Elephant ear), *Sagittaria lancifolia* (Bulltongue), *Hydrilla verticillata* (Water thyme),

Eichhornia crassipes (Water hyacinth), *Panicum hemitomon* (Maidencane), and *Salix nigra* (Black willow) (Delaune and Jugsujinda 2003).

The sampling strategy was designed to facilitate the comparison of IPT denitrification rates among benthic and marsh habitats, as well as across two different water temperatures. Near-field and far-field locations were selected based on water temperature, salinity and NO_3^- concentration data which indicated both variables were similar among the two locations. Thus, the near-field and far-field locations allowed for comparison among the two habitats and water temperatures while incorporating local spatial variation into the denitrification estimates.

The four sampling locations included two freshwater lakes and two freshwater marsh sites located ca. 4.3 km (near-field) and ca. 6.6 km (far-field) downestuary from the Caernarvon diversion structure. The two freshwater lakes were: near-field, Big Mar Lake (B-NF, 29°49.436'N & 89°53.941'W), and far-field, Lake Leary (B-FF, 29°47.913'N & 89°52.381'W). The two freshwater marsh sites were: near-field (M-NF, 29°49.353'N & 89°54.597'W) and far-field (M-FF, 29°48.132'N & 89°54.997'W) from the diversion structure in upper Breton Sound estuary (Fig. 4). The B-NF and B-FF refer to benthic (B) habitat sites and M-NF and M-FF refer to marsh (M) habitat sites.

Isotope Pairing Technique and Analysis

The foundation of the IPT relies on the stable nitrogen isotopes which vary greatly in their natural abundance, 99.64% ^{14}N and 0.36% ^{15}N. In IPT experiments $^{15}NO_3^-$ molecules are added to the water column where they can either diffuse into the sediment and be directly denitrified (direct denitrification), or mix with ambient $^{14}NO_3^-$, produced via nitrification, within the sediment interstitial water and be denitrified (coupled nitrification-denitrification). Denitrification from this isotope mixture, or from direct denitrification, can produce N_2

molecules with atomic molecular masses 28, 29 and 30 (Steingruber et al. 2001). In ambient atmosphere, the $^{29}N_2$ and $^{30}N_2$ atomic masses only constitute 0.7299 and 0.001%, respectively, of the entire atmospheric N_2 pool (Nielsen 1992). To calculate denitrification rates the dissolved $^{28}N_2$ concentration was determined using an empirical formula which incorporates temperature, salinity and multiple N_2 solubility coefficients (Weiss 1970; Hamme and Emerson 2004). Denitrification rates were estimated from the ^{15}N isotope gas production following previously derived equations (Nielsen 1992; Steingruber et al. 2001).

In the experiments with no initial $^{14}NO_3^-$ concentrations the added $^{15}NO_3^-$ in the water column is not diluted, and therefore D_{15} represents the $^{15}NO_3^-$ direct denitrification rate and D_n is the coupled nitrification-denitrification rate (Nielsen 1992; Steingruber et al. 2001). All data presented below from the current study is D_{15} and D_n. Total denitrification activity is assumed to be the sum of D_{15} and D_n.

The IPT requires four assumptions be fulfilled for proper determination of denitrification rates. To test these assumptions, water-sediment cores are exposed to different $^{15}NO_3^-$ concentrations (as $K^{15}NO_3^-$, minimum 98% ^{15}N) which are collected over time. This experimental approach, known as a time-series experimental set-up, was performed during both IPT assumption experiments (Fig. 6A) and water temperature experiments (Fig. 6B) under dark conditions. The advantage to this approach is it allows for the monitoring of the $^{29}N_2$ and $^{30}N_2$ production over time (Steingruber et al. 2001). The disadvantage is only one denitrification rate is obtained per $^{15}NO_3^-$ incubation concentration, and this one denitrification rate is based on eight different sediment cores, which will certainly have heterogeneity among them (Steingruber et al. 2001).

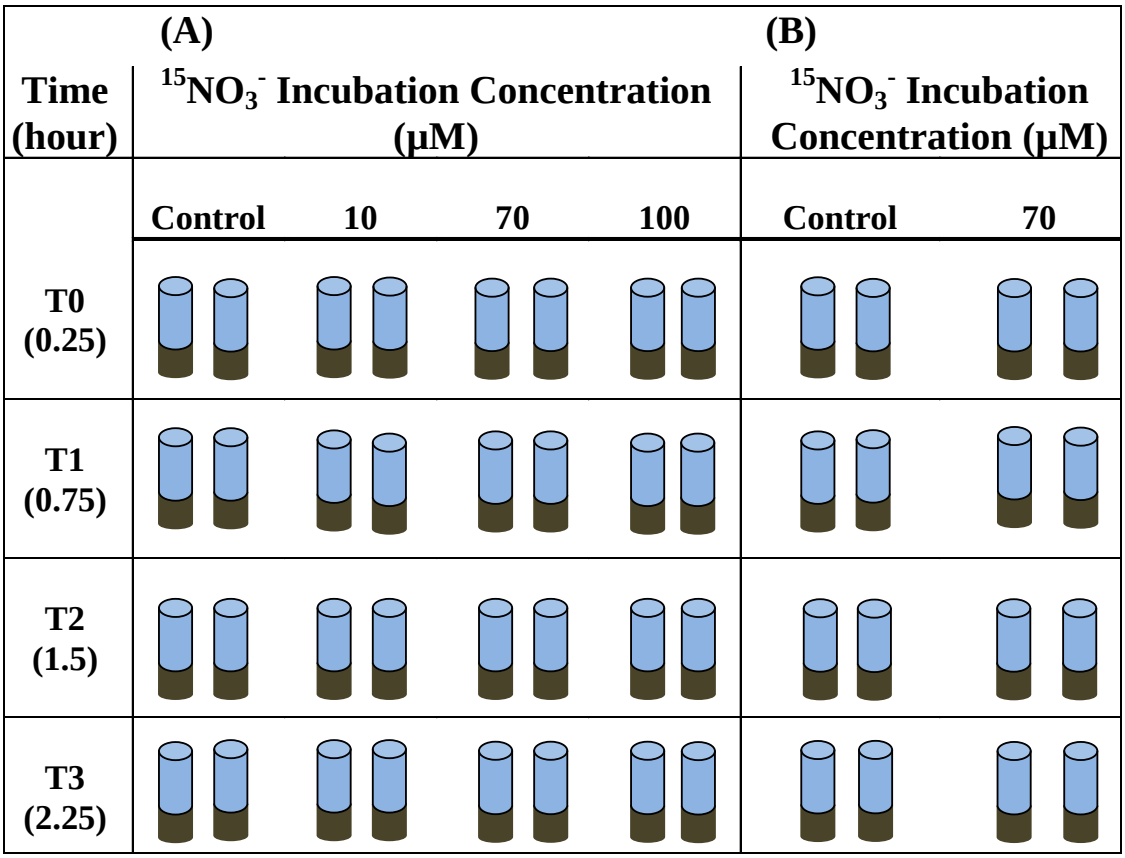

Fig. 6 IPT experimental approaches, assumption experiment approach (A), and water temperature experiment approach (B).

Field Sampling

Intact sediment cores were collected to evaluate IPT assumptions under varying habitat and temperature conditions using clear PVC tubing (2.1 cm i.d.) on several dates at the benthic (B-NF and B-FF) and marsh (M-NF and M-FF) locations. The first IPT test was performed at the B-NF site on November 2006, and then the marsh NF and FF were assessed on the same day in February 2007. Sampling at the B-FF site occurred in July 2007. Finally, B-NF and M-NF sites were evaluated on the same day in February 2008. In addition to the IPT assumption experiments just described, the influence of water temperature on denitrification at each of four

locations (B-NF, B-FF, M-NF and M-FF) was evaluated on three separate occasions in July/August 2007, February/March 2008, and April 2008 (water temperature experiments).

Approximately 7-10 cm of sediment was collected using the clear PVC tubing, and the top 2-3 cm were transferred intact into glass cores (2.1 cm i.d. x 12 cm length). Parafilm and a rubber stopper were securely fit to the glass core bottom, and sediment samples were transported back to the laboratory under dark conditions. Water from Big Mar Lake represents Mississippi River water diverted through the Caernarvon diversion structure and was collected and used as pre-incubation (14-17 h) water prior to experimentation. Discharge from the Caernarvon diversion ranged from 28.3-221.2 $m^3 s^{-1}$ during all sampling occasions with the highest discharge occurring during the February 2008 samplings. Water depth varied during sampling due to fluctuations in the Caernarvon freshwater discharge rate; 0.8-2.0 m depth for B-NF and B-FF, M-NF from saturated soil to 0.7 m depth and M-FF from saturated soil to 0.3 m depth. Big Mar Lake water temperature ranged from 6.9-29.9 °C and dissolved oxygen concentrations ranged from 6.1-10.2 mg L^{-1} during all sampling occasions. Surface water salinity on all occasions never exceeded 1.0 ppt. An aliquot of water from Big Mar Lake was used for nutrient analysis.

Laboratory Incubation

Intact sediment cores were incubated overnight (14-17 h) under dark conditions, prior to experimentation, using filtered water collected from Big Mar Lake. To prevent anoxic conditions, oxygen was bubbled into the surface water of the cores. Oxygen and temperature were monitored during pre-incubation and prior to $^{15}NO_3^-$ addition the following day. Overlying water column oxygen measurements (data not shown) were on average > 95% saturation and water temperature did not fluctuate > ± 2 °C from 22 °C during pre-incubations. IPT assumption experiments were performed first to evaluate the assumptions of the IPT for the four study

locations (Fig. 6A). The IPT assumption experiments were performed at 22 °C. Following the IPT assumption experiments, water temperature experiments were performed to evaluate the influence of water temperature and habitat type on denitrification (Fig. 6B). A summary of experimental conditions during IPT assumption and water temperature experiments, including ambient NO_3^- concentrations and the direct and coupled denitrification rates obtained during each experiment, are presented in Appendix D.

The initial IPT assumption experiment in November 2006 at the B-NF location was done using a control (no $^{15}NO_3^-$) and four different $^{15}NO_3^-$ incubation concentrations (2, 15, 60 and 100 µM $K^{15}NO_3^-$, minimum 98% ^{15}N) over four time periods (0, 1, 2 and 4 hours) for a total of 40 experimental cores. November 2006 final incubation solutions contained ambient $^{14}NO_3^-$ from Big Mar Lake plus the $^{15}NO_3^-$ additions; which for example, the 60 µM $^{15}NO_3^-$ incubation solution had a final total ($^{14}NO_3^- + {}^{15}NO_3^-$) incubation concentration of 110.4 µM (48.3 $^{14}NO_3^-$ + 62.1 $^{15}NO_3^-$) (Appendix D). The proceeding IPT assumption experiments in February 2007 at the M-NF and M-FF locations were modified from the November 2006 experiment by using a control (no $^{15}NO_3^-$) and only three different total ($^{14}NO_3^- + {}^{15}NO_3^-$) incubation concentrations (10, 70 and 100 µM) as well as decreasing the total experiment duration (0.25, 0.75, 1.5 and 2.25 hours) for a total of 32 cores per experiment. Unlike the November 2006 experiment, February 2007 incubation solutions were adjusted by first decreasing the ambient $^{14}NO_3^-$ concentration prior to $^{15}NO_3^-$ addition to achieve the final target concentration of 70 µM $^{15}NO_3^-$ (35 µM $^{14}NO_3^-$ + 35 µM $^{15}NO_3^-$) (Appendix D).

After reviewing the data from the above experiments an additional modification was made to the final two IPT assumption experiments in February 2008 at the M-NF and B-NF locations. Incubation solutions were prepared using distilled deionized water instead of site

water from Big Mar Lake (Appendix D), thus removing $^{14}NO_3^-$ from the overlying water. Nielsen, 1992 used this same experimental approach by only using $^{15}NO_3^-$ incubation concentrations. These experimental approaches still applied a control (no $^{15}NO_3^-$) and three different $^{15}NO_3^-$ incubation concentrations (30, 70 and 200 µM $K^{15}NO_3^-$, minimum 98% ^{15}N) over four time periods (0.25, 0.75, 1.5 and 2.25 hours) for a total of 32 cores per experiment.

Water temperature experiments were incubated with a control (no $^{15}NO_3^-$) and one $^{15}NO_3^-$ incubation concentration (70 µM $K^{15}NO_3^-$, minimum 98% ^{15}N) over four time periods (0.25, 0.75, 1.5 and 2.25 hours) for a total of 32 cores, 16 cores per incubation temperature treatment (8 and 22 °C). All water temperature incubation solutions were prepared with distilled deionized water instead of site water from Big Mar Lake (Appendix D). Three water temperature experiments were performed at each site (B-NF, B-FF, M-NF and M-FF) for both incubation water temperature treatments. The mean (± 1 SE) target $^{15}NO_3^-$ (µM) concentrations for all locations during IPT assumption and water temperature experiments ranged from 68.0 to 74.8 µM $^{15}NO_3^-$ (Appendix D).

The water columns from the experimental cores were not mechanically stirred with stir bars; instead, they were oscillated in an incubation system at a low velocity (30 rpm) to avoid disrupting the sediment-water interface. The incubation system also maintained constant temperature, and was designed to facilitate $^{15}NO_3^-$ diffusion into the sediment. To control water column temperature a cold water circulation system was developed to maintain an 8 °C incubation temperature. An ice chest with flexible tubing cycled water from the incubation system through a motorcycle radiator and back to the incubation system while maintaining the desired 8 °C incubation water temperature.

Prior to adding the $^{15}NO_3^-$ incubation solutions, overlying water (i.e. pre-incubation water) was withdrawn from the cores without disturbing the sediment-water interface. Sediment cores were re-filled with a known $^{15}NO_3^-$ incubation solution and the core tops were capped with parafilm and a fabricated screw cap. Sediment cores were filled to avoid any headspace. Two cores per enrichment concentration were collected every 45 minutes starting at 0.25 h (15 min) after $^{15}NO_3^-$ was added to each individual core. The sediment NO_3^- vertical profile was assumed to be established and stable after 20 minutes (Nielsen and Glud 1996).

At each sampling time, two cores per enrichment concentration were removed from the incubation system, the core cap was unscrewed and the core was stirred to homogenize the water column and sediment. Cores were then re-capped and shaken by hand for three minutes to allow for any N_2 trapped in the sediment to diffuse into the water-phase. Previous experiments indicate > 70% of the N_2 produced by the denitrification process can become trapped in the sediment (Reddy et al. 1989; Lohse et al. 1996; Nielsen and Glud 1996; Steingruber et al. 2001; Herrman and White 2008). After hand shaking, the cores were centrifuged at about 2500 rpm for 4.5 min to separate sediment and water. The rubber septum was then removed and 20 ml of sample water was collected with a gas tight 60 ml syringe. Under a helium (He) atmosphere 20 ml ultra pure He gas was drawn into the gas tight syringe containing the 20 ml of sample water. The syringe was shaken by hand for three minutes to promote N_2 diffusion from the water into the He headspace. Previous research indicates approximately 98% of the N_2 gas will diffuse from the water into the He headspace during this shaking (Steingruber et al. 2001). At this point, the gas tight syringe contains 20 ml sample water and a 20 ml mixture of He and N_2. After shaking was complete the gas tight syringe was brought back under the He atmosphere, where 10 ml of the He and N_2 mixture was discarded, and the remaining 10 ml was transferred into 10 ml glass

vacutainers (BD Vacutainer Serum, BD, Franklin Lakes, NJ, USA). The glass vacutainers were then refrigerated for later analysis of $^{15}N_2$ gas. The remaining 20 ml water sample was filtered, collected, and frozen for nutrient analysis.

Sediment, Nutrient and Gas Analysis

Sediment physical characteristics were measured on composite samples collected from the top 2-3 cm at all four locations. Soil composition was determined using the hydrometer method (Patrick 1958). Sediment cores (2.1 cm i.d. x 12 cm length) collected for bulk density determination were oven-dried at 60 °C to a constant weight (Blake and Hartge 1986). After drying, sediments from the top 2-3 cm were ground and homogenized for total nitrogen (N) and carbon (C) content by a 4010 elemental analyzer combustion system (Costech Analytical Technologies, Inc. Valencia, CA, USA). Total organic matter was measured by loss on ignition at 550 °C for two hours (Davies 1974; Aspila et al. 1976). Surface water nutrient concentrations, ammonium (NH_4^+), nitrite (NO_2^-), NO_3^- and phosphate (PO_4^{-3}), collected from the sampling locations and obtained from sediment core incubations were determined colorimetrically using a Flow IV Autoanalyzer (OI Analytical, College Station, TX, USA). Collected headspace gas samples were analyzed for $^{14}N^{15}N$ and $^{15}N^{15}N$ abundance and concentration using a gas chromatograph coupled to a triple-collector 20/20 isotope ratio mass spectrometer (GC-MS, RoboPrep-G plus, Europa Scientific, SerCon Ltd, UK).

Statistical Analysis

To evaluate the IPT assumptions, linear regressions were performed between direct denitrification (D_{15}), coupled denitrification (D_n) and increasing water column $^{15}NO_3^-$ concentrations (SAS 2004). Nineteen total IPT assumption denitrification observations were pooled together to perform regressions (Appendix D). Regressions were also performed by

pooling observations by habitat type (i.e. benthic and marsh). An exploratory analysis was performed on the data to identify any extreme outliers which may have resulted from "a biogeochemical hot spot or hot moment" (Mcclain et al. 2003), sediment heterogeneity or other environmental conditions prior to linear regressions. Only three outliers were identified and were not included in the linear regressions (see Benthic Near-Field exp. March, 2007 Appendix D). An additional three IPT assumption observations were not included in linear regressions because the experiment was performed at 10 °C (Appendix D). Linear regressions from IPT assumption experiments between $^{29}N_2$-N, $^{30}N_2$-N excess (i.e. amount after control core $^{29}N_2$ and $^{30}N_2$ production was removed) production and time were evaluated by each experiment at all four locations (SAS 2004).

A split plot ANOVA design was applied using SAS PROC MIXED to test for differences in denitrification rates among four experimental factors: date, location, habitat and water temperature. Date was considered a random effect, and location, habitat and water temperature were fixed effects. The date was assigned a categorical level: 1, 2 or 3. The fixed factors had two levels: location (near-field and far-field), habitat (marsh and benthic) and water temperature (8 and 22 °C). Interaction among factors was considered for all analyses. Pairwise comparisons were performed with Tukey's HSD test when significant differences ($p < 0.05$) were observed within a main effect or interaction. Normality and homoscedasticity assumptions were not statistically relevant for this particular design and were not tested because only one observation (denitrification rate) was generated for each date, location, habitat and water temperature treatment combination.

Results and Discussion

Isotope Pairing Technique Assumptions

Accurate determination of in-situ denitrification rates using the IPT requires four key assumptions be fulfilled. Three of the four IPT assumptions were evaluated, however the fourth IPT assumption, the influence of annamox, was not evaluated. As a result denitrification rates may be overestimated as a portion of the evolved N_2 gas may have been produced via the annamox process instead of through the denitrification process (Eyre et al. 2002).

$^{15}NO_3^-$ Addition

$^{15}NO_3^-$ additions must not modify the coupled denitrification (D_n) rate based on ambient $^{14}NO_3^-$ concentrations (Nielsen 1992; Rysgaard et al. 1995; Lohse et al. 1996; Steingruber et al. 2001; Eyre et al. 2002). All nineteen IPT assumption observations were pooled together to test this assumption. Direct denitrification (D_{15}) was significantly correlated ($R^2 = 0.21$, n = 19, p = 0.05) with increasing $^{15}NO_3^-$ concentrations (2-200 µM) (Fig. 7), and D_n was independent of $^{15}NO_3^-$ concentrations indicating the first assumption was fulfilled.

Further D_{15} regressions versus $^{15}NO_3^-$ concentrations were evaluated by pooling observations by habitat type (i.e. benthic and marsh). When evaluated by habitat type, D_{15} regressions versus increasing $^{15}NO_3^-$ concentrations was significantly correlated ($R^2 = 0.62$, n = 10, p = 0.007) (Fig. 8), and D_n was independent of $^{15}NO_3^-$ concentrations indicating the first assumption was fulfilled at the benthic habitat. D_{15} was not, however, significantly correlated with increasing $^{15}NO_3^-$ concentrations at the marsh habitat ($R^2 = 0.22$, n = 9, p = 0.20) (Fig. 9). However, D_n did remain independent of $^{15}NO_3^-$ concentrations indicating the first assumption was also fulfilled at the marsh habitat. The observed D_{15} linearity from all nineteen IPT

assumption and pooled benthic habitat observations indicates denitrification from in-situ NO_3^- sources was not obstructed by $^{15}NO_3^-$ additions, and therefore, the $^{15}NO_3^-$ addition assumption was fulfilled. The non-significant correlation in the marsh habitat suggests denitrification from in-situ NO_3^- sources may have been obstructed by $^{15}NO_3^-$ additions within the concentration range applied. However, since D_n did remain independent of $^{15}NO_3^-$ additions the assumption was fulfilled (Nielsen and Glud 1996).

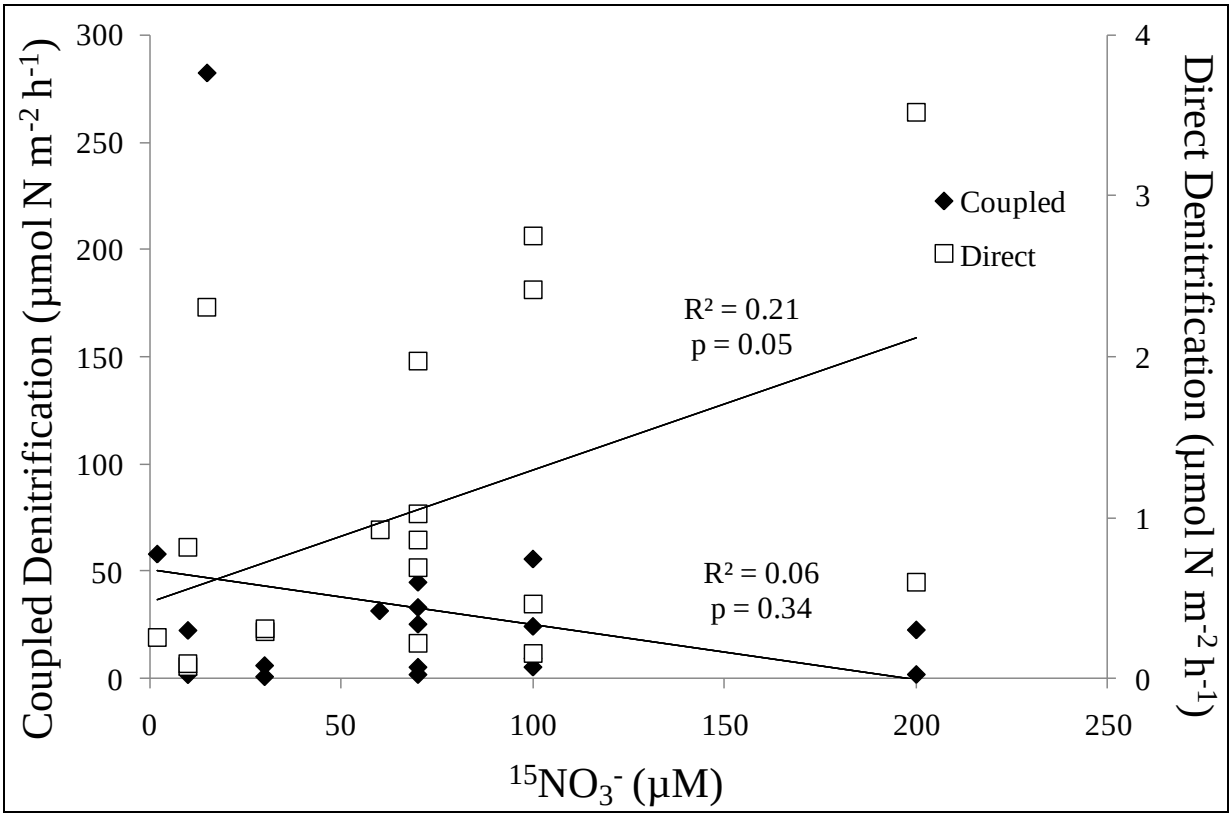

Fig. 7 IPT assumption experiments (n = 19) pooled from all sampling locations. Coupled nitrification-denitrification (D_n) and direct denitrification (D_{15}) (µmol N m^{-2} h^{-1}) as a function of increasing water column $^{15}NO_3^-$ concentrations (2-200 µM) for experiments performed November 2006-April 2008.

Homogenous Isotope Mixing

A second assumption is $^{15}NO_3^-$ additions must mix homogenously with the $^{14}NO_3^-$ pool in the water column and the sediment interstitial water (Nielsen 1992; Rysgaard et al. 1995; Lohse

et al. 1996; Steingruber et al. 2001; Eyre et al. 2002). In theory, homogenous isotope mixing will never reach "ideal" conditions in static cores (Groffman et al. 2006). Phenomena such as diffusion barriers, sediment heterogeneity, bioturbation, isotope fractionation and in-homogenous nitrification activity will create local deviations in the $^{14}NO_3^-$ and $^{15}NO_3^-$ transport to the sediment denitrification zone, which in turn can result in underestimating denitrification activity (Nielsen 1992; Rysgaard et al. 1995; Steingruber et al. 2001; Eyre et al. 2002).

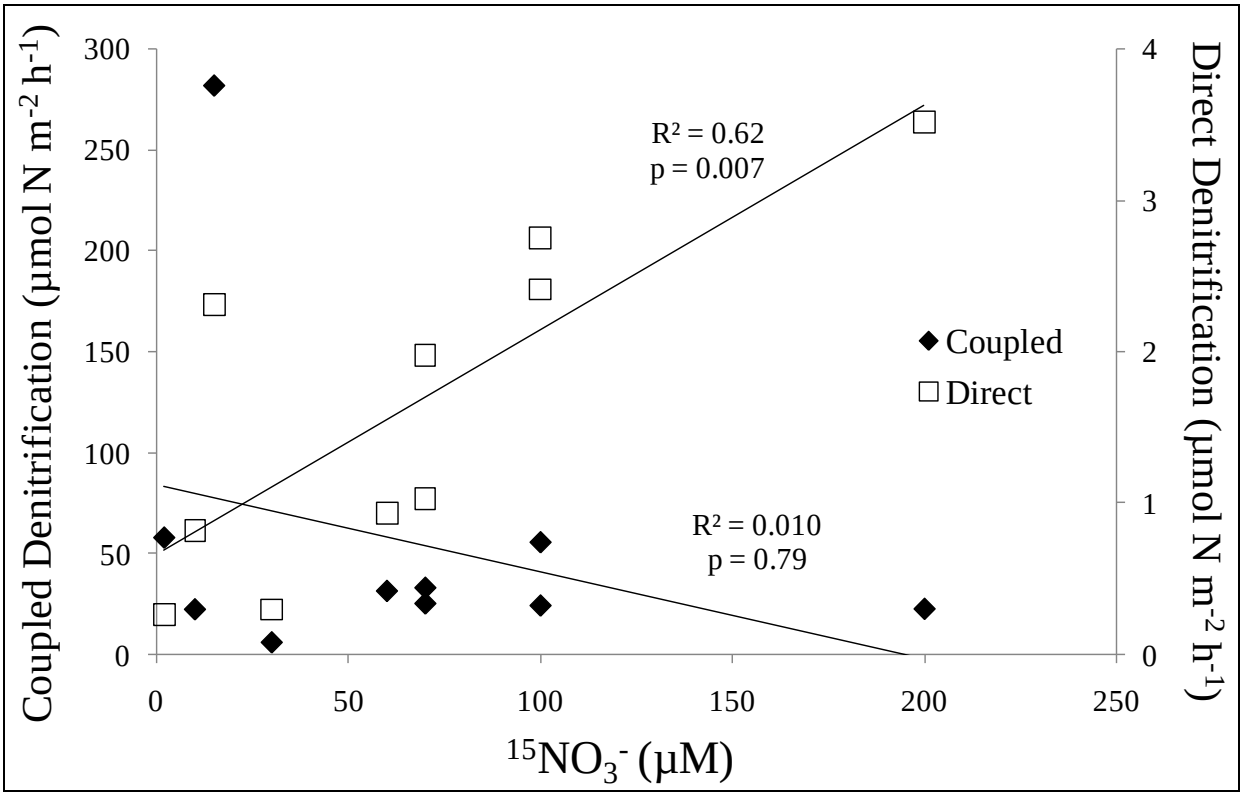

Fig. 8 Benthic IPT assumption experiments (n = 10). Coupled nitrification-denitrification (D_n) and direct denitrification (D_{15}) (µmol N m^{-2} h^{-1}) as a function of increasing water column $^{15}NO_3^-$ concentrations (2-200 µM) for experiments performed November 2006-April 2008.

To evaluate the homogenous isotope mixing assumption sediment cores can be incubated under different $^{15}NO_3^-$ concentrations. If D_n is independent from increasing $^{15}NO_3^-$ concentrations then the homogenous mixing assumption is fulfilled. If D_n is linearly correlated

with $^{15}NO_3^-$ concentrations this suggests inhomogeneous isotope mixing is occurring. Coupled denitrification (D_n) rates from all nineteen IPT assumption observations were pooled together to test this assumption, and the results indicate D_n was not significantly ($R^2 = 0.06$, n = 19, p = 0.34) related to the water phase $^{15}NO_3^-$ concentration (Fig. 7). This suggests the two isotopes (in the form $^{14}NO_3^-$ and $^{15}NO_3^-$) were homogenously mixed during experimentation and thus the assumption was fulfilled.

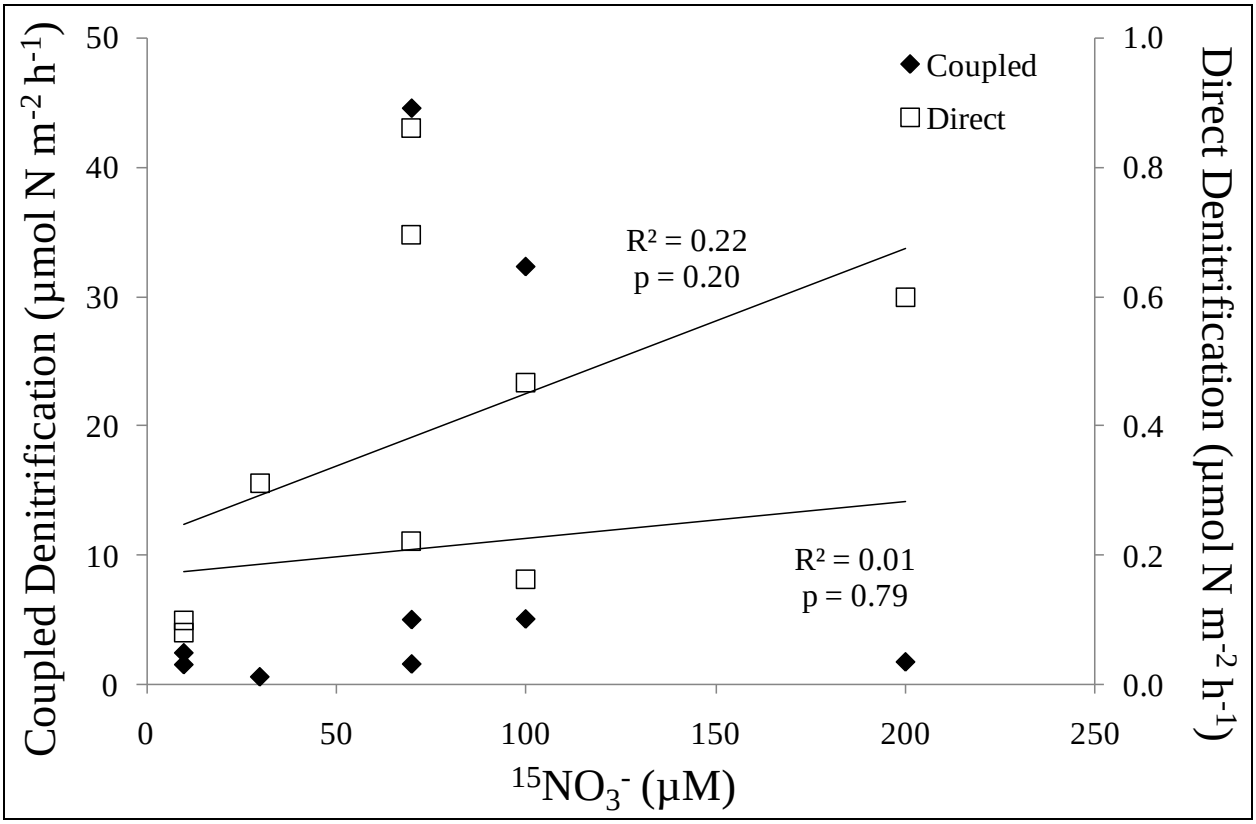

Fig. 9 Marsh IPT assumption experiments (n = 9). Coupled nitrification-denitrification (D_n) and direct denitrification (D_{15}) (µmol N m^{-2} h^{-1}) as a function of increasing water column $^{15}NO_3^-$ concentrations (2-200 µM) for experiments performed November 2006-April 2008.

Further regressions with D_n versus $^{15}NO_3^-$ concentrations were evaluated by pooling observations by habitat type (i.e. benthic and marsh). When evaluated by habitat, D_n regressions versus increasing $^{15}NO_3^-$ concentrations was not significantly correlated at the benthic habitat (R^2

= 0.010, n = 10, p = 0.37) (Fig. 8). Similarly, D_n was not significantly correlated with increasing $^{15}NO_3^-$ concentrations at the marsh habitat (R^2 = 0.010, n = 9, p = 0.79) (Fig. 9). This suggests the two isotopes (in the form $^{14}NO_3^-$ and $^{15}NO_3^-$) were also homogenously mixed when evaluated by habitat. This further supports the homogenous isotope mixing assumption within the water column and sediment interstitial water, and suggests the incubation system developed for my IPT experiments provides non-static conditions while maintaining intact sediment core structure.

Stable NO_3^- Concentration Gradient

A third assumption is a stable NO_3^- concentration gradient must become established across the sediment-water interface shortly after $^{15}NO_3^-$ addition. If this gradient does not become established the lack in available $^{15}NO_3^-$ will lead to an underestimation in denitrification activity (Nielsen 1992; Rysgaard et al. 1995; Steingruber et al. 2001; Eyre et al. 2002). To test this, experiments were conducted by sacrificing individual cores over time to assess whether a linear N_2 gas production was occurring.

The initial experiment from B-NF in November 2006 (Fig. 10A) at 110.5 µM incubation concentration mixture ($^{14}NO_3^-$ & $^{15}NO_3^-$) did show a significant correlation for $^{29}N_2$ (R^2 = 0.999, n = 2, p = 0.02), and a correlation, however not significant, for $^{30}N_2$ (R^2 = 0.991, n = 2, p = 0.06) through the initial two hour incubation. However, incubations to four hours had correlations which were not significant for $^{29}N_2$ (R^2 = 0.32, n = 4, p = 0.43) and for $^{30}N_2$ (R^2 = 0.29, n = 4, p = 0.46) (Fig. 10A). It was thought oxygen became limiting by the fourth hour in these experimental cores, and as a result all future experiments were reduced to two hours and fifteen minutes (as described in the methods (Fig. 6) and laboratory incubation section) to decrease the possibility for oxygen depletion within the water-sediment experimental core. A following

experiment at B-NF in February 2008 showed a significant correlation for $^{29}N_2$ and $^{30}N_2$ due to the decrease in experiment duration from four hours to two hours and fifteen minutes (Fig. 10B).

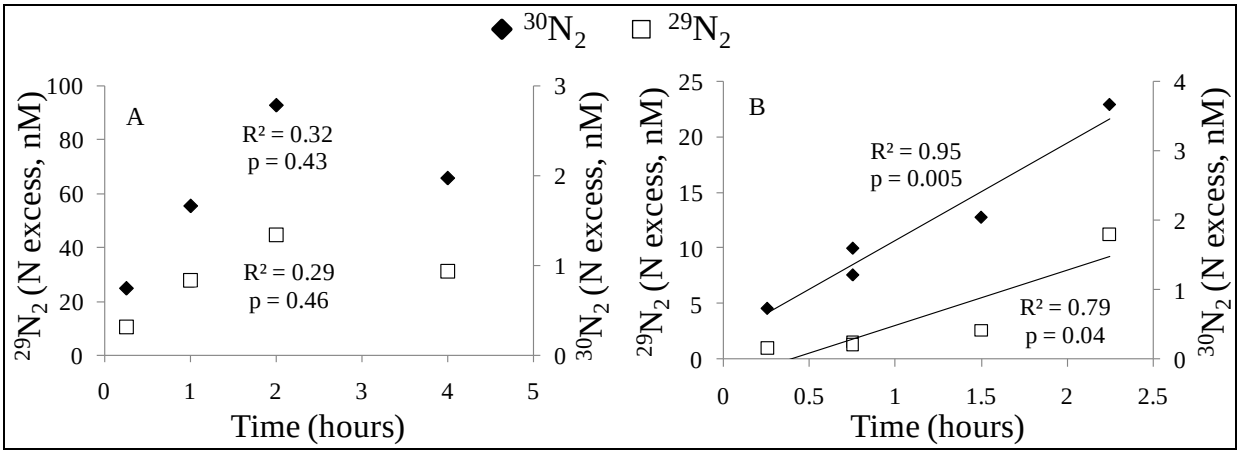

Fig. 10 Production of $^{29}N_2$ and $^{30}N_2$ (N excess, nM) over time (hours) after $^{15}NO_3^-$ addition: (A) B-NF, 11/06; (B) B-NF, 02/08. Primary axis represents $^{29}N_2$ and secondary axis represents $^{30}N_2$. Figure (A) based on 110.5 µM incubation mixture ($^{14}NO_3^-$ & $^{15}NO_3^-$) and (B) is based on 71.3 µM $^{15}NO_3^-$ incubation concentration.

The B-NF experiment from November 2006 (Fig. 10A) illustrates the preferential production of $^{29}N_2$ relative to $^{30}N_2$ due to the ($^{14}NO_3^-$ & $^{15}NO_3^-$) incubation mixture. As a result, the ensuing B-NF experimental approach from February 2008 (Fig. 10B) was modified by removing the $^{14}NO_3^-$ from the water column and incubating with only a $^{15}NO_3^-$ enrichment solution. This resulted in a decreased production of $^{29}N_2$ relative to the $^{30}N_2$ product (Fig. 10A vs. 10B). This same preferential production in $^{29}N_2$ relative to $^{30}N_2$ is also illustrated in the M-NF experiment from February 2007 (Fig. 11A) which had a ($^{14}NO_3^-$ & $^{15}NO_3^-$) incubation concentration mixture. As a result, the ensuing M-NF experimental approach from February 2008 (Fig. 11B) was modified by applying only a $^{15}NO_3^-$ enrichment solution, and this resulted in a decreased production of $^{29}N_2$ relative to the $^{30}N_2$ product. Comparing the two B-NF experiments (Fig. 10A and 10B) and two M-NF experiments (Fig. 11A & 11B) illustrates the $^{29}N_2$ production did decrease and $^{30}N_2$ production increased, which I suggest resulted from

eliminating the high ambient $^{14}NO_3^-$ and $^{14}NH_4^+$ sources from within the water column. At higher $^{15}NO_3^-$ incubation concentrations more $^{14}NO_3^-$ is trapped and measured directly as $^{29}N_2$, which can potentially result in an in-situ denitrification rate miscalculation (Nielsen 1992).

All remaining experiments, both IPT assumption and water temperature experiments, showed significant correlations in increasing $^{29}N_2$ and $^{30}N_2$ production over time, which was a direct result of the two modifications to the experimental approach: 1) reducing the experiment duration from four hours to two hours and fifteen minutes, and 2) removing the ambient $^{14}NO_3^-$ and $^{14}NH_4^+$ from the overlying water and only using a $^{15}NO_3^-$ incubation concentration.

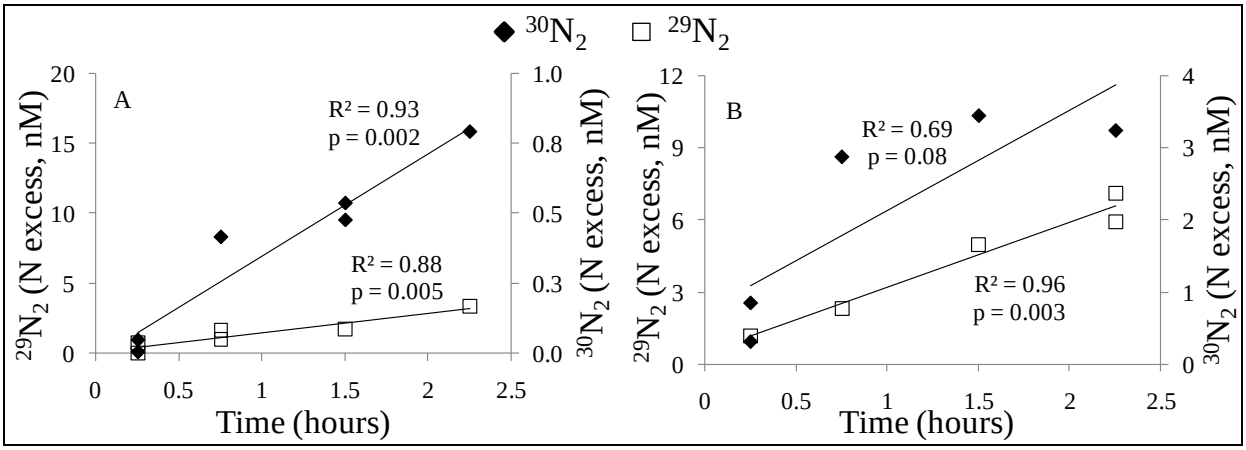

Fig. 11 Production of $^{29}N_2$ and $^{30}N_2$ (N excess, nM) over time (hours) after $^{15}NO_3^-$ addition: (A) M-NF, 02/07; (B) M-NF, 02/08. Primary axis represents $^{29}N_2$ and secondary axis represents $^{30}N_2$. Figure (A) based on 101.1 µM incubation mixture ($^{14}NO_3^-$ & $^{15}NO_3^-$) and (B) is based on 71.3 µM $^{15}NO_3^-$ incubation concentration.

In summary, three of the four IPT assumptions were evaluated, but the fourth IPT assumption, the influence of annamox, was not evaluated. Annamox is a microbial process performed by chemolithoautotrophic bacteria where ammonium (NH_4^+) is oxidized under anoxic conditions with nitrite (NO_2^- or NO_3^-) acting as the terminal electron acceptor resulting in N_2 formation as the end product (Mulder et al. 1995; Van De Graaf et al. 1995). The anammox process has yet to be investigated in coastal Louisiana freshwater environments. Due to the

potential for anammox activity in Louisiana freshwater sediments, denitrification rates presented here could potentially be overestimated as a portion of the evolved N_2 gas may have been derived from the anammox process instead of denitrification (Eyre et al. 2002). However, a recent anammox-denitrification study from New England freshwater marshes found anammox contributed less than 3% towards the total N_2 production measured (Koop-Jakobsen and Giblin 2009).

The $^{15}NO_3^-$ addition assumption was fulfilled for all nineteen observations pooled. When observations were pooled by habitat, the benthic habitat fulfilled the $^{15}NO_3^-$ assumption; however, there was a non-significant correlation between direct denitrification (D_{15}) and increasing $^{15}NO_3^-$ concentrations in the marsh habitat. This suggests coupled denitrification from in-situ NO_3^- sources may have been obstructed by $^{15}NO_3^-$ additions within the concentration range applied. This result may suggest greater sediment heterogeneity within the marsh habitat versus benthic habitat sediments since direct denitrification (D_{15}) was not significantly correlated to increasing $^{15}NO_3^-$ concentrations. However, since coupled denitrification (D_n) remained independent of $^{15}NO_3^-$ additions, the $^{15}NO_3^-$ addition assumption was still fulfilled for the marsh habitats. The homogenous isotope mixing assumption was fulfilled for all nineteen observations pooled as well as for both habitat treatments. The stable NO_3^- concentration gradient assumption was fulfilled for all four locations. However, two manipulations to the IPT experimental approach, after the November 2006 and February 2007 experiments were performed which included: 1) a decrease in the incubation time from four hours to two hours and fifteen minutes to prevent the establishment of anoxic conditions, and 2) a removal of $^{14}NO_3^-$ from the water column to increase the $^{30}N_2$ product and decrease the $^{29}N_2$ product. Both manipulations appeared

to aide in the fulfillment of IPT assumptions which resulted in correct in-situ denitrification rate determination.

Coupled vs. Direct Denitrification Activity

A direct denitrification (D_{15}) and coupled nitrification-denitrification (D_n) summary (Appendix D) for all experiments performed during the current study, including ambient NO_3^- concentrations and $^{15}NO_3^-$ enrichment concentrations, are presented for the sampling period November 2006 through April 2008. Coupled nitrification-denitrification (D_n) activity accounted for 85% of the total denitrification activity in the benthic and marsh habitats during all (IPT and water temperature) experiments when incubated at 22 °C with $^{15}NO_3^-$ incubation concentrations ranging from 5 to 200 µM. However, D_n did decrease by 44% and 40% in benthic and marsh habitats when water temperature decreased from 22 to 8 °C (Fig. 12). This decrease in D_n activity, as water temperature decreased, may suggest nitrifying bacteria in upper Breton Sound were influenced by fluctuations in temperature, similar to what has been observed in other studies (Henriksen and Kemp 1988). The optimum temperature for pure cultured nitrifying bacteria ranges from 25 to 35 °C (Focht and Verstraete, 1977). Conversely, a very broad temperature range (15 to 75 °C) has been observed for denitrifying bacteria and the denitrification process (Focht and Verstraete 1977). It does appear nitrifying bacteria were more sensitive to fluctuations in water temperature than denitrifying bacteria, since for both the benthic and marsh habitats coupled denitrification decreased with water temperature (Fig. 12) Conversely, direct denitrification did not appear to be influenced by changes in overlying water temperature (Fig. 12). However, without isolating the different bacterial groups, and exposing them individually to the two different temperature treatments with similar substrate conditions, it

is hard to conclude whether water temperature influenced denitrifying or nitrifying bacteria in the current study.

Coupled nitrification-denitrification (D_n) activity appeared to be influenced by habitat with greater D_n rates in the benthic vs. the marsh habitat (Fig. 12). This result may have been due to greater sediment heterogeneity, inhomogeneous nitrification activity, or because only surficial sediments were collected, thus omitting coupled nitrification-denitrification activity occurring around marsh plant rhizospheres found deeper in the sediments (Nielsen 1992; Welsh et al. 2000; Steingruber et al. 2001). Previous research suggests coupled nitrification-denitrification estimates are enhanced around intertidal macrophytes and their associated rhizosphere compared to nonvegetated sediments. These researchers also indicated enhanced coupled nitrification-denitrification rates surrounding the intertidal macrophytes rhizosphere at 1 to 20 cm depths versus the surficial 0 to 1 cm sediment layer (Christensen and Sorensen 1986; Reddy et al. 1989). Therefore, the marsh habitat sediments from upper Breton Sound provide a dilemma for the current IPT design; $^{15}NO_3^-$ diffusion will not reach deep into the plant rhizosphere where optimal conditions persist for coupled nitrification-denitrification activity.

Similar limitations of the IPT have been observed in other marsh and plant colonized environments. For example, seagrass-colonized sediments can have a strong influence on denitrification activity (Iizumi et al. 1980; Kristensen 1988) through oxygen excretion by seagrass roots, which creates a rhizosphere micro-environment conducive to coupled nitrification-denitrification activity (Henriksen and Kemp 1988; Caffrey and Kemp 1990; Caffrey and Kemp 1992). This may cause the IPT to underestimate coupled nitrification-denitrification (D_n) because this process is occurring away from the $^{15}NO_3^-$ diffusion zone (Welsh et al. 2000). Consequently, D_n estimates from marsh habitat sediments in the current study may

reflect the denitrification process occurring only in surficial sediments, and thus not including D_n associated with the marsh rhizosphere (Nielsen 1992), since only the top 2 to 3 cm sediment layer was incubated. However, previous IPT investigations (with experimental core dimensions ranging from 3.4 to 20 cm i.d., 8 to 15 cm sediment and 10 to 25 cm overlying water, with 30 µM $^{15}NO_3^-$ enrichment) from other marsh habitats also indicate low direct and coupled nitrification-denitrification rates similar to my results (Rysgaard et al. 1996; Trimmer et al. 2000; Welsh et al. 2000).

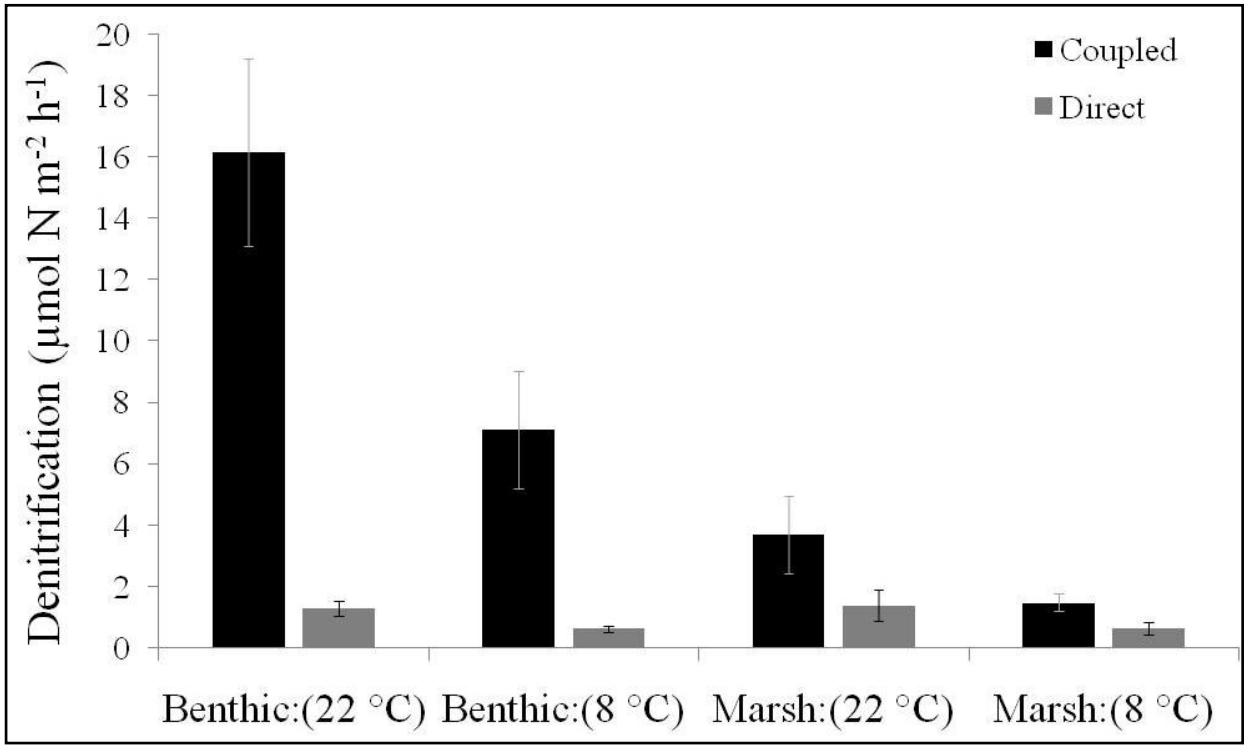

Fig. 12 Mean (± 1 SE) coupled and direct denitrification rates (µmol N m^{-2} h^{-1}) from benthic and marsh habitats at each water temperature treatment (n = 6) from upper Breton Sound estuary, Louisiana. Denitrification rates presented are from approximately 70 µM $^{15}NO_3^-$ incubation solutions.

Mean direct denitrification (D_{15}) rates never exceeded 2 µmol N m^{-2} h^{-1} for both habitats incubated at 22 and 8 °C (Fig. 12). Direct denitrification (D_{15}) did not appear to be influenced by

water temperature or habitat (Fig. 12). During these same experiments, $^{15}NO_3^-$ flux into the sediment, within the experimental cores, was calculated to be -387.5 (± 105.7) and -107.2 (± 60.4) μmol N m^{-2} h^{-1} for the marsh and benthic habitats, respectively. These high $^{15}NO_3^-$ flux rates into the sediment suggest there was no physical or chemical limitation preventing $^{15}NO_3^-$ from reaching the sediment denitrification zone. The low direct denitrification rates also suggest direct denitrification was not the predominant process removing $^{15}NO_3^-$ within the experimental cores. These direct denitrification results are contrary to previous investigations in upper Breton Sound, which observed significant removal of Mississippi River NO_3^- from the overlying water by either benthic (Delaune and Jugsujinda 2003; Lane et al. 1999) or marsh (Bond 2006) habitats. For example, NO_2^- + NO_3^- removal efficiencies of 88 to 97% were calculated for benthic habitats in Breton Sound (Lane et al. 1999). Considering the small spatial scale under which all denitrification estimates were obtained, direct denitrification appears to be a negligible process for removing Mississippi River NO_3^- from the overlying water column, and suggests other nitrogen pathways (i.e. plant assimilation, microbial assimilation, DNRA, etc.) are responsible for the large NO_2^- + NO_3^- uptake rates previously observed.

Water Temperature and Habitat Type

Mean total denitrification (direct + coupled denitrification) activity in upper Breton Sound was significantly different among habitat and water temperature treatments (Table 2). The other spatial effect, location, had no significant effect on denitrification activity (Table 2). However, there was a significant interaction between habitat and water temperature effects (Table 2; Fig. 13). The benthic habitat at 22 °C had a significantly higher mean (± 1 SE) denitrification rate (17.5 ± 3.1 μmol N m^{-2} h^{-1}) than at 8 °C (7.8 ± 1.9 μmol N m^{-2} h^{-1}) (Fig. 13). The benthic habitat mean (± 1 SE) denitrification rate at 22 °C was also significantly higher than

the marsh habitat (5.1 ± 1.5, 2.1 ± 0.45 µmol N m^{-2} h^{-1}) at 22 and 8 °C (Fig. 13). These results suggest water temperature has a significant influence on denitrification activity in benthic habitats; however, it did not have a significant effect on denitrification activity in marsh habitats at the temperatures applied (Fig. 13). Total denitrification activity decreased in the benthic (56%) and marsh (58%) habitats due to a decrease in water temperature when incubated at 22 versus 8 °C, and the majority of this change in total denitrification activity was predominantly due to a decrease in coupled nitrification-denitrification rather than direct denitrification (Fig. 12).

Table 2 Statistical results of spatial and water temperature effects on denitrification rates measured in upper Breton Sound, Louisiana. Temp = water temperature. Significant levels are indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ns = not significant.

Effect	df	F	p
Location	1, 8	0.51	ns
Habitat	1, 8	11.63	**
Location x Habitat	1, 8	0.12	ns
Temp	1, 8	18.83	**
Location x Temp	1, 8	0.87	ns
Habitat x Temp	1, 8	5.35	*
Location x Habitat x Temp	1, 8	0.03	ns

Similar water temperature effects among different habitat types were observed in New England marshes, where the denitrifying community responded more to increased water temperature in benthic habitats versus drier marsh habitats (Kaplan et al. 1979). Observed denitrification measurements using the N_2 gas flux method from Narragansett Bay, Rhode Island, Boston Harbour, Massachusetts, and the Pawcatuck River Estuary, Rhode Island ranged from 0 to 195 µmol N_2 m^{-2} h^{-1} for all three systems studied (Nowicki 1994). The denitrification rates, from all three systems, increased exponentially as water temperature increased from 5 to 20 °C during incubations (Nowicki 1994). Similarly, seasonal denitrification estimates in benthic

sediments from Boston Harbor were highest during the high temperature (206 µmol N_2 m^{-2} h^{-1} at 18 °C) seasons and lowest during the low temperature (< 5 µmol N_2 m^{-2} h^{-1} at 1.5 °C) seasons (Nowicki 1994; Nowicki et al. 1997).

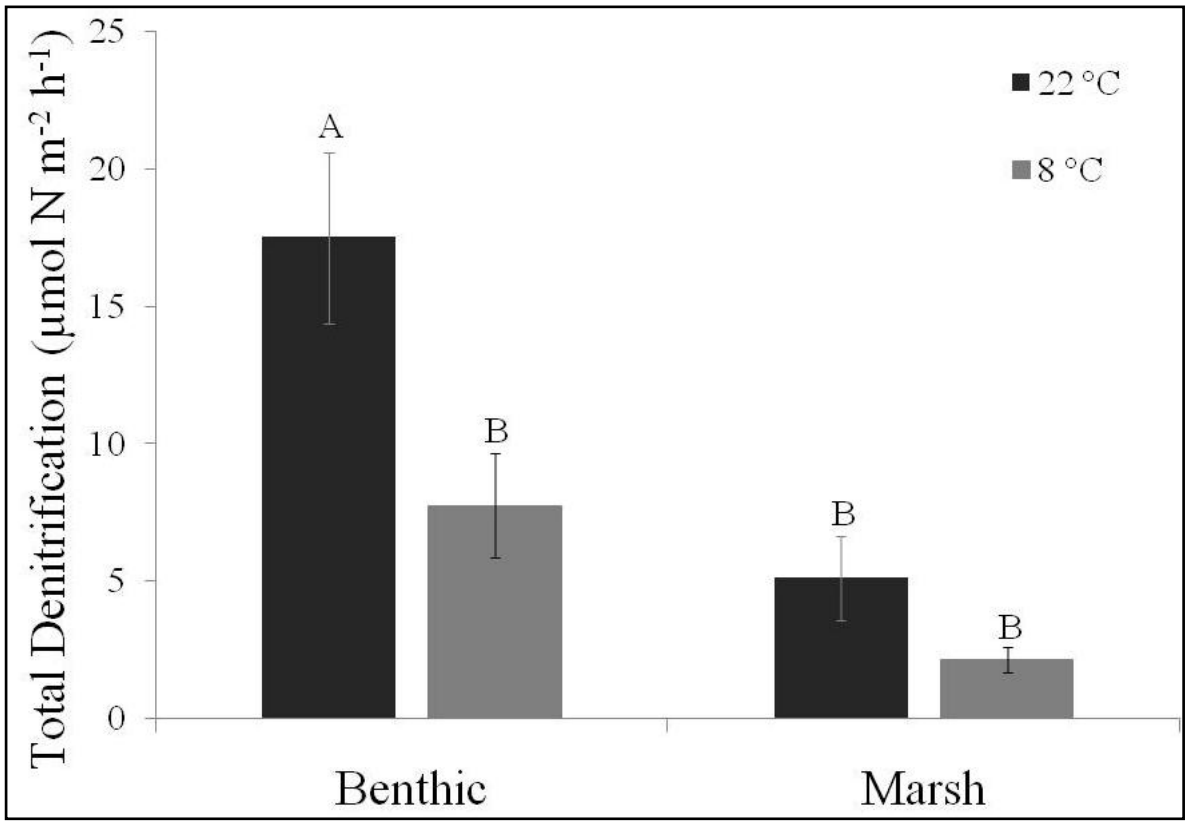

Fig. 13 Mean (± 1 SE) total denitrification rate (µmol N m^{-2} h^{-1}) measured during water temperature experiments at both habitats (benthic and marsh) in upper Breton Sound, Louisiana. Different capital letters indicate a significant difference ($p < 0.05$) among denitrification rates. Data was pooled by habitat and temperature for this analysis (n = 6). Denitrification rates based on approximately 70 µM $^{15}NO_3^-$ incubation solutions.

Locally, water temperature can have a strong influence on denitrification in areas receiving high NO_3^- inputs; (i.e. Mississippi River water diversions and the Atchafalaya River basin) when NO_3^- concentrations are highest (> 100 µM), the Mississippi River water temperature is low (< 8 °C). Denitrification activity, measured using the acetylene reduction technique, increased as water temperature increased (from a high of 77.2 µmol N m^{-2} h^{-1} at 8 °C

to 289.6 µmol N m^{-2} h^{-1} at 30 °C) within the Atchafalaya River basin in Louisiana (Lindau et al. 2008). The researchers also observed a rapid response in peak denitrification activity over their experiment duration as temperature increased from 8 to 22 - 30 °C (Lindau et al. 2008). Their data, as well as data from my study, suggest the denitrifying communities in Louisiana freshwater ecosystems are very responsive to NO_3^- inputs, and fluctuations in water temperature can regulate denitrification activity by influencing the spatial or temporal distribution of nitrifying and denitrifying bacterial populations (Lindau et al. 2008).

Sediment and Water Quality Parameters

Select sediment characteristics from all four locations indicated similarities among the two near-field and far-field benthic and the two near-field and far-field marsh habitat sites (Table 3). These similarities between the near-field and far-field benthic and marsh habitats allowed for the two benthic and marsh habitats to be used as replicates during denitrification analyses while incorporating spatial variability. Sediment characteristics also indicate differences between benthic and marsh habitats (Table 3). The lower bulk density (g cm^{-3}) in the marsh habitats, compared to the benthic habitats, suggests marsh sediments may provide more favorable conditions for denitrification. A lower bulk density indicates greater sediment pore space, which provides a favorable diffusion path for NO_3^- to diffuse into the denitrification zone (Reddy and Delaune 2008). However, greater pore space could also limit denitrification by providing less substrate for denitrifying bacteria to colonize. Percent organic matter and total carbon (g m^{-2}) were also higher in the marsh than in the benthic habitats, and these two sediment characteristics favor denitrification by providing more electron donor substrate, and more colonization area for denitrifying bacteria (Koike and Hattori 1978; Twilley and Kemp 1986; Seitzinger 1988; Herbert and Nedwell 1990; Sorenson and Revsbech 1990). These properties (lower bulk density, greater

total carbon and higher percent organic matter) suggest more favorable conditions for the denitrification process in marsh habitats. However, on average lower denitrification rates were observed in the marsh habitat then in the benthic habitat, which suggests these sediment characteristics may not explain the difference in denitrification estimates between the two habitats.

Table 3 Select sediment characteristics from the top two to three centimeters of sediment. OM = organic matter; B.D. = bulk density; C:N = atomic carbon: nitrogen ratio; Total C = total carbon; Total N = total nitrogen.

							Soil Composition		
Habitat	**Location**	**OM (%)**	**B.D. ($g\ cm^{-3}$)**	**C:N ratio**	**Total C ($g\ m^{-2}$)**	**Total N ($g\ m^{-2}$)**	**% Sand**	**% Silt**	**% Clay**
Marsh	Near-Field	20 ±0.97	0.39 ±0.06	12.3 ±0.37	620.4 ±56.7	58.6 ±4.5	20	44	36
	Far-Field	51 ±8.0	0.16 ±0.02	14.2 ±0.45	820.2 ±155.0	67.3 ±12.8	nd	nd	nd
Benthic	Near-Field	7 ±0.29	0.58 ±0.04	10.6 ±0.21	196.4 ±18.2	21.6 ±2.1	5	78	17
	Far-Field	10 ±0.61	0.39 ±0.05	12.2 ±0.32	228.0 ±32.4	21.8 ±2.7	12	59	30

M-FF soil composition not determined (nd) due to high percent organic matter. All remaining sites soil composition determined on composite sample (n = 1). Percent organic matter (OM) based on (n = 3) for M-FF site, three remaining sites (n = 7). All other sediment characteristics from all sites based on (n = 7). Standard deviation indicated by (±).

Big Mar Lake water temperature during all sampling events (November 2006-April 2008) ranged from 7.7 to 30.4 °C with a mean (± 1 SE) water temperature 18.3 (± 0.17) °C. Mean (± 1 SE) in-situ nutrient concentrations (NO_2^-, NO_3^-, NH_4^+ and PO_4^{-3}) from Big Mar Lake during all sampling events (November 2006-April 2008) were 0.90 (± 0.17), 96.03 (± 11.92), 9.91 (± 1.19) and 3.34 (± 0.46) µM, respectively. Mean (± 1 SE) in-situ porewater nutrient concentrations (NO_2^-, NO_3^-, NH_4^+ and PO_4^{-3}) from the marsh habitats (July 2007-April 2008) were 0.29 (± 0.11), 1.82 (± 1.17), 546.1 (± 171.0) and 57.6 (± 20.9) µM, respectively.

Isotope Pairing Technique Perspective

Global IPT Perspective

This was the first IPT study conducted in Louisiana. To make a comparison to other IPT studies, I selected denitrification rates from the literature which were based on the $^{15}NO_3^-$ incubation concentration applied. The $^{15}NO_3^-$ incubation concentration for the current estimate was 70 µM and the remaining studies $^{15}NO_3^-$ concentration ranged from 19 to 85 µM. Studies were grouped into two categories: benthic and marsh. Benthic environments ranged from saline bays (Nielsen and Glud 1996; Eyre et al. 2002), to a seagrass meadow (Welsh et al. 2002), a freshwater stream (Nielsen 1992) and North Sea continental shelf sediment (Lohse et al. 1996). Marsh environments chosen included a saltwater marsh (Trimmer et al. 2000) and a freshwater marsh receiving agricultural runoff (Herrman and White 2008). Total denitrification (direct + coupled) (µmol N m^{-2} h^{-1}) estimates from the current study are very comparable to the other IPT denitrification estimates (Fig. 14). This comparison does suggest the IPT approach presented in the current study can provide reliable denitrification estimates for benthic and marsh habitat sediments based on previous IPT rates from other environmental systems. Considering the differences in experimental approaches (core dimensions, experiment duration, intact cores vs.

sediment slurries) among these studies (Fig. 14), and the low variability observed in denitrification estimates, suggests the IPT could be an optimum choice for performing cross system analyses of denitrification activity. These cross system analyses could improve our understanding of how significant denitrification may be in the global nitrogen cycle.

Louisiana Denitrification Perspective

Total denitrification (direct + coupled denitrification) activity from all IPT experiments in my study in upper Breton Sound estuary ranged from 0.28 to 284.1 μmol N m^{-2} h^{-1} (Table 4). There is a need for better collaboration in Louisiana denitrification investigations, since comparing IPT denitrification estimates with others (Table 4) is futile considering the wide range of experimental conditions applied. Nevertheless, future goals should be to compare different denitrification techniques throughout different habitats of coastal Louisiana; however, it is critical to apply similar experimental conditions (i.e. experiment duration, NO_3^- enrichment concentrations, intact sediment cores vs. sediment slurries, lab vs. field, etc) to minimize the variability currently present in denitrification estimates. This variability in denitrification estimates, especially between different techniques (Table 4), is certainly one obstacle currently limiting our understanding of the significance denitrification plays in nutrient budgets from coastal ecosystems (Groffman et al. 2006).

The denitrification rates obtained from my study are comparable to denitrification rates generated using other nitrogen isotope techniques in coastal Louisiana (Iwai 2002; Miao et al. 2006; Yu et al. 2006). Experiments from Lake Cataouatche, a lake which receives diverted Mississippi River water from the Davis pond freshwater diversion, show similar denitrification rates as measured in my study. For example, using the ^{15}N dilution technique in Lake Cataouatche, denitrification rates were 47.5 and 56.2 μmol N m^{-2} h^{-1} for fringe and interior

freshwater benthic sediments over a 57-day period and a 107 µM $^{15}NO_3^-$ and 107 µM $^{14}NO_3^-$ enrichment (Miao et al. 2006). In a second example from Lake Cataouatche, denitrification rates ranged from 9.8 to 47.6 µmol N m^{-2} h^{-1} over a 29-day incubation period and a 3571.4 µM $^{15}NO_3^-$ enrichment using the $^{15}N_2$ gas emission technique (Iwai 2002). Estimates from these two benthic studies are comparable to the estimates for benthic habitats obtained in my study, and suggest these techniques may provide consistent results for comparisons among different benthic systems. It is also interesting to note that even under extremely enriched NO_3^- conditions (Iwai 2002) and long incubation periods (Miao et al. 2006) the IPT and the two other nitrogen isotope techniques provide comparable denitrification estimates.

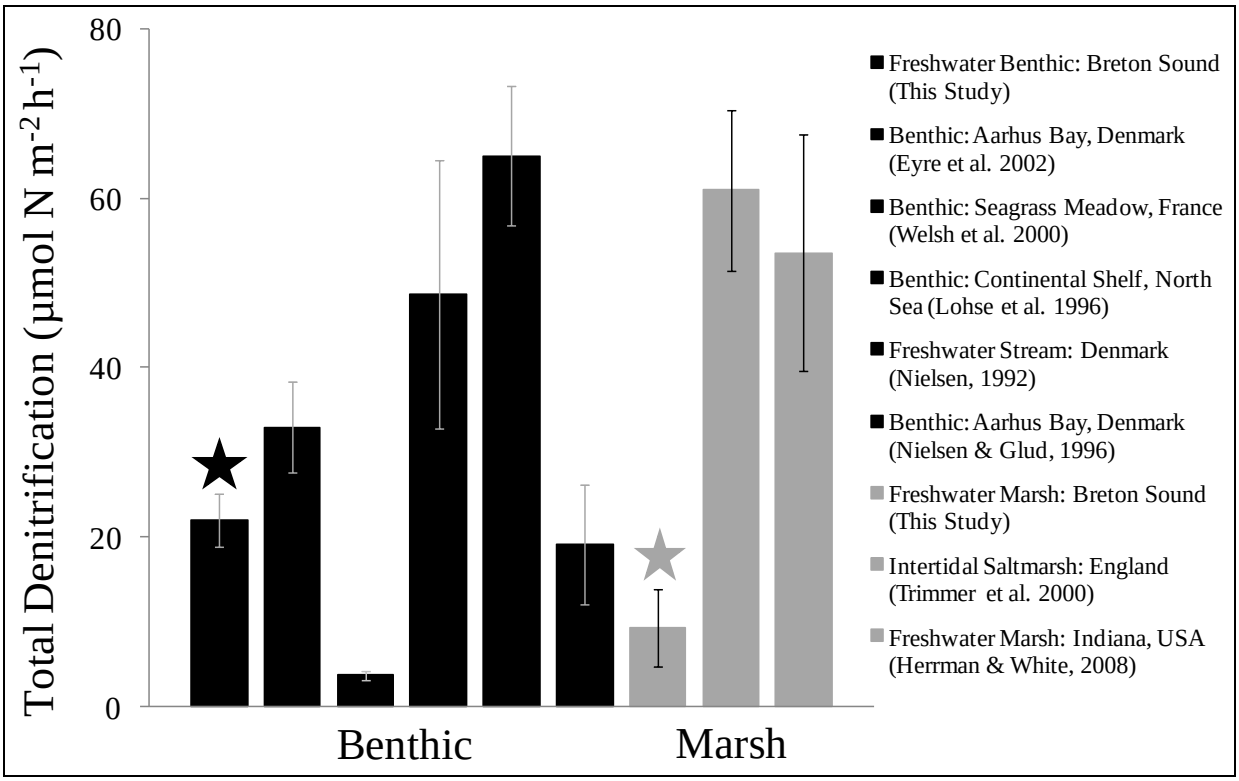

Fig. 14 Mean total IPT denitrification rates (µmol N m^{-2} h^{-1}) for benthic and marsh habitats from various locations around the world. Black and gray star denote the current studies denitrification estimates from the benthic and marsh habitat, respectively. The current study denitrification estimates (n = 6) based on a 70 µM $^{15}NO_3^-$ concentration, and the remaining studies ranged from 19 to 85 µM $^{15}NO_3^-$.

Considering another individual study from a comparable marsh habitat in Davis pond, which applied a static chamber to measure denitrification in-situ, denitrification rates ranged from 0.0 to 678.9 µmol N m^{-2} h^{-1} over a 13 day period based on a 3800 mg m^{-2} $^{15}NO_3^-$ enrichment (Yu et al. 2006). The IPT denitrification estimates for the marsh habitat in my study, based on a 3 to 300 mg N m^{-2} $^{15}NO_3^-$ enrichment, are low and range from 0.65 to 45.4 µmol N m^{-2} h^{-1}. The difference in denitrification activity between my study and Yu et al. 2006 could be explained by the different enrichments applied, or by the sampling approach. My study focused on the top 2 to 3 cm sediment layer, and Yu et al. 2006 focused over a greater sediment depth profile of 1 to 30 cm. As mentioned previously, research suggests coupled nitrification-denitrification estimates are enhanced around intertidal macrophytes and their associated rhizosphere compared to nonvegetated sediments. These researchers also indicated enhanced coupled nitrification-denitrification rates surrounding the intertidal macrophytes rhizosphere at 1 to 20 cm depths versus the surficial 0 to 1 cm sediment layer (Christensen and Sorensen 1986; Reddy et al. 1989).

Breton Sound Perspective

The only published potential denitrification estimates from the Caernarvon river diversion outfall region, Big Mar Lake (benthic habitat), used the acetylene inhibition technique (Delaune and Jugsujinda 2003). Their denitrification estimates range from 13.7 to 199.5 (± 6.9/53.3) µmol N m^{-2} h^{-1} for a 1750 mg m^{-2} enrichment, and from 41.9 to 349.8 (± 2.2/6.5) µmol N m^{-2} h^{-1} with a 3500 mg m^{-2} over an 11 day experiment. Disappearance of water column NO_3^- was estimated at 288.7 and 506.0 µmol N m^{-2} h^{-1} with a 1750 and 3500 mg m^{-2} enrichment, respectively, over a 16 day experiment. N_2O emissions from these sediments were relatively

minor and only accounted for about 1.5% of the applied NO_3^-. Total denitrification estimates from the benthic habitats in the current study ranged from 6.0 to 284.1 µmol N m^{-2} h^{-1} for a 3 to 300 mg N m^{-2} enrichment. Considering the two different techniques applied, which have their inherent differences, and the broad range in NO_3^- enrichment strategies, the two studies did provide comparable denitrification ranges with low associated variability.

Table 4 Range in denitrification rates for many different ecosystems throughout coastal Louisiana. Ranges were grouped by denitrification techniques and represent published studies from 1981-2009. Isotope pairing technique total denitrification rates (direct + coupled) presented are from the current study based on 2 to 200 µM $^{15}NO_3^-$ incubation concentrations (after Rivera-Monroy et al. 2009, in review).

Denitrification Technique	**Denitrification (µmol N m^{-2} h^{-1})**
Indirect Techniques	21 – 2157.7
Acetylene Inhibition Technique	0.18 – 1338.6
^{15}N Techniques	2.7 – 2852.8
Isotope Pairing Technique	0.28 – 284.1

Total denitrification rates from the marsh habitats in my study, considering all enrichments 2 to 200 $^{15}NO_3^-$ µM, were considerably lower than the benthic habitat estimates. Total denitrification rates ranged from 0.65 to 45.4 µmol N m^{-2} h^{-1}, and the majority of this rate was a result of coupled denitrification. In a NO_3^- flux study from upper Breton Sound marshes, mean NO_3^- flux into the sediment was -65 µmol N m^{-2} h^{-1} with ambient NO_3^- concentrations of 60 µM (Twilley and Bond 2001, unpublished). Mean direct denitrification rates from the current study in the marsh habitat, 1.02 (± 0.28) µmol N m^{-2} h^{-1}, imply direct denitrification is not a major pathway for NO_3^- removal during my experiments under the small spatial scale examined.

These results suggest other NO_3^- removal pathways (i.e. plant assimilation, microbial assimilation, DNRA, etc.) in my experiments must be responsible for the difference between the NO_3^- flux and the direct denitrification rate measured.

Conclusions

This study did demonstrate robust denitrification estimates may be obtained using the IPT in a relatively straight forward and timely manner. This study was the first to employ the IPT in coastal Louisiana oligohaline ecosystems. However, much experimentation is still needed to strengthen the IPT's implementation (e.g. quantifying annamox) before extrapolating denitrification rates up to the ecosystem level. IPT assumptions and denitrification estimates indicate benthic and marsh habitat sediments from upper Breton Sound are applicable for IPT implementation. The IPT assumptions appeared to be fulfilled more rigorously in benthic habitats than in marsh habitats. The non-significant correlation between direct denitrification (D_{15}) and increasing $^{15}NO_3^-$ concentrations for the marsh habitat suggests coupled denitrification may have been obstructed by $^{15}NO_3^-$ additions within the concentration range applied. However, since coupled denitrification (D_n) remained independent from $^{15}NO_3^-$ additions, the $^{15}NO_3^-$ addition assumption was fulfilled for the marsh habitat. Water temperature does appear to be a statistically significant environmental control on denitrification estimates from benthic habitats in upper Breton Sound; however, water temperature is less significant in controlling denitrification estimates in marsh habitats.

The majority of total denitrification measured in benthic and marsh habitats was a result of coupled denitrification vs. direct denitrification. Direct denitrification estimates were very low indicating benthic and marsh habitats will remove only a small percentage of Mississippi

River NO_3^- entering the Breton Sound estuary, implying other nitrogen cycling processes (i.e. plant assimilation, phytoplankton uptake, microbial assimilation, DNRA or NO_3^- dilution) may dominate in NO_3^- removal. However, a relatively small spatial scale was sampled in this study with the objective to focus on and fulfill the IPT assumptions, and not necessarily evaluate Mississippi River NO_3^- disappearance across the Breton Sound estuary.

The marsh habitat sediments from upper Breton Sound do provide a dilemma for the current IPT design; $^{15}NO_3^-$ diffusion will not reach deep into the plant rhizosphere where optimal conditions persist for coupled nitrification-denitrification activity. This observation suggests the current IPT experimental design should be modified prior to future marsh habitat experiments so that a coupled nitrification-denitrification estimate incorporating the plant rhizosphere may be obtained. The consequence of this $^{15}NO_3^-$ diffusion limitation into the denitrification zone will be an underestimation in coupled nitrification-denitrification rates when applying the IPT to surficial marsh habitat sediments. Therefore, the total denitrification ($D_n + D_{15}$) estimates from the marsh habitats in the current study are presumed to be underestimated. I do believe the current IPT experimental design is what contributed to the low total and direct denitrification estimates in the marsh habitats. However, I do believe the benthic coupled and direct denitrification estimates from the IPT experimental design are accurate.

More Mississippi River freshwater diversions are planned for coastal Louisiana in the future with the notion they are important restoration tools for delaying or impeding coastal marsh degradation. However, constructing more Mississippi River freshwater diversions may be delayed or restricted due to the potential negative impacts on water quality created by the low water temperature and high NO_3^- load of the Mississippi River during high river stages. My study did indicate cold (8 °C) Mississippi River water diverted through the Caernarvon diversion

structure into receiving benthic habitats has a significant influence on denitrification activity. This cold Mississippi River water caused a 56% and 58% denitrification reduction at benthic and marsh habitats, respectively, as compared to denitrification estimates obtained at 22 °C. During these periods when cold Mississippi River water is diverted denitrification activity may become suppressed allowing NO_3^- to propagate further downstream into nitrogen limiting ecosystems. This NO_3^- propagation could result in eutrophication or the development of harmful algal blooms in downstream water bodies (Nixon et al. 1996; Howarth et al. 2000; Galloway et al. 2002; Howarth et al. 2002; Vitousek et al. 2002; Wissel et al. 2005; Twilley and Rivera-Monroy 2009).

Coastal Louisiana is annually vulnerable to periodic tropical storm activity. The influence this storm activity can have on coastal landscapes, like upper Breton Sound, can be detrimental. Hurricanes and tropical storms can supply coastal Louisiana with valuable inorganic sediment (Turner et al. 2006; Day et al. 2007). However, these same storms (ex. hurricane's Katrina and Rita) have also converted fragile coastal wetlands into open water ecosystems (Barras 2007; Day et al. 2007). To compound the matter, my observations from upper Breton Sound suggest post-hurricane management activities can further alter the natural environment by modifying diverted Mississippi River water flow. This post-hurricane management activity has the objective to maintain navigable waters by dredging waterways and applying this dredged material on adjacent marshes creating spoil banks, which essentially impounds the marsh habitat and prevents overland marsh flow. This makes dispersing cold diverted nutrient rich Mississippi River water over marsh and benthic habitats more difficult. These spoil banks prevent diverted Mississippi River water from entering into marsh habitats and they also decrease water residence time in benthic habitats by channelizing water flow. These effects combined will further erode the upper estuaries denitrification potential. Therefore,

quantifying denitrification in dynamic environments (i.e. Breton Sound) over greater temporal/spatial scales, including prior to and after tropical storm disturbances, and in the context of percent Mississippi River NO_3^- removed, will provide important nitrogen cycling information currently missing for guiding coastal restoration objectives in Louisiana (Twilley and Rivera-Monroy 2009). Understanding how all environmental controls act collectively across the landscape and over time to influence nitrogen cycling processes will be very important for implementing future freshwater diversion projects in Louisiana. As scientists we are challenged by these novel ecological processes with great aspiration to provide scientifically robust discoveries to government officials, environmental managers and citizens so they can understand the impacts future freshwater diversions will have in coastal Louisiana.

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APPENDIX A. DENITRIFICATION RATES ESTIMATED USING ^{15}N ISOTOPE TECHNIQUES

Appendix A. Denitrification rates estimated using ^{15}N isotopic techniques in several ecosystems throughout coastal Louisiana for the period 1981-2009. For superscripts see key ID below table. (Modified from Rivera-Monroy et al. 2009, in review)

Ecosystem [A]	Habitat [B]	Experiment Duration Location [C]	[^{15}N] Enrichment [D]	Technique [E]	Rate (μmol N m^{-2} h^{-1})	Reference
Bayou Chevrieu (1)	(2)	27 day (2)	10 NO_3 (3)	(1)	413.2 - 829.7	(Lindau et al. 1988a)
Bayou Chevrieu (1)	(2)	27 day (2)	10 NH_4 (3)	(1)	601.5 - 898.9	(Lindau et al. 1988a)
St. James Parish (1)	(2)	32 day (1)	10 NO_3 & NH_4 (3)	(2)	383.7 - 1579.6	(Delaune et al. 1998)
St. James Parish (1)	(2)	32 day (1)	10 NO_3 (3)	(1)	66.6 – 335.0 (243 +/- 31.2)	(Delaune et al. 1998)
St. James Parish (1)	(2)	32 day (1)	10NH_4 (3)	(1)	4.8 – 1488.0 (79.1 +/- 20.4)	(Delaune et al. 1998)
Lake Cataouatche (1)	Fringe (1_b)	57 day (1)	107 NO_3 & $^{14}NO_3$ (1)	(2)	56.2 (± 45.7)	(Miao et al. 2006)
Lake Cataouatche (1)	Middle (1_b)	57 day (1)	107 NO_3 & $^{14}NO_3$ (1)	(2)	47.5 (± 31.6)	(Miao et al. 2006)
Lac des Allemands (1)	(5)	46 day (3)	10 NO_3 (3)	(1)	803.2	(Lindau et al. 1991)
Lac des Allemands (1)	(5)	46 day (3)	20 NO_3 (3)	(1)	1020.8	(Lindau et al. 1991)
Lac des Allemands (1)	(5)	46 day (3)	30 NO_3 (3)	(1)	1336.3	(Lindau et al. 1991)
Lac des Allemands (1)	(5)	46 day (3)	10 NH_4 (3)	(1)	4.5	(Lindau et al. 1991)
Lac des Allemands (1)	(5)	46 day (3)	20 NH_4 (3)	(1)	193.5	(Lindau et al. 1991)
Lac des Allemands (1)	(5)	46 day (3)	30 NH_4 (3)	(1)	321.3	(Lindau et al. 1991)

(**Appendix A.** continued)

Ecosystem [A]	Habitat [B]	Experiment Duration Location [C]	[^{15}N] Enrichment [D]	Technique [E]	Rate (µmol N m^{-2} h^{-1})	Reference
Davis Pond (1)	(5)	13 day (2)	3.8 NO_3 (3)	(3)	0 - 678.6 (±79.9)	(Yu et al. 2006)
East of Leeville, LA (1)	(4)	33 day (3)	10 NO_3 & NH_4 (3)	(1)	28.9 - 395.6 (±144 / 33.9)	(Lindau and Delaune 1991)
Lake Cataouatche (1)	(1_b)	29 day (1)	806 NO_3 (1)	(1)	9.8 - 47.6(±35.7 / 15.1)	(Iwai 2002)
Lac Des Allemands (1)	(1_b)	142 day (1)	.143 N (1)	(2)	64.0 - 66.1 (65)	(Delaune and Lindau 1989)
Little Lake (1)	(1_c)	95 day (1)	.143 N (1)	(2)	71.5 - 76.9 (74.2)	(Delaune and Lindau 1989)
Lac Des Allemands (1)	(1_b)	16 week (1)	50 NH_4 (2)	(4)	44.8	(Smith and Delaune 1983b)
Airplane Lake (1)	(1_a)	16 week (1)	50 NH_4 (2)	(4)	11.4	(Smith and Delaune 1983b)
Lake Verret (2)	(1_b)	48 day (1)	126 NO_3 (1)	(2)	114 - 154 (±8 / 10)	(Delaune and Smith 1987)
Spring Bayou WMA, Avoyelles Parish (4)	(3)	67 day (2)	10 NO_3 (3)	(1)	92.2	(Lindau et al. 1994)
Spring Bayou WMA, Avoyelles Parish (4)	(3)	67 day (2)	30 NO_3 (3)	(1)	182.9	(Lindau et al. 1994)
Crowley, LA (5)	(6_a)	35 – 153 day (1)	24.2 NO_3 (1)	(2)	101 (± 12)	(Lindau et al. 1988b)
Crowley, LA (5)	(6_a)	35 – 153 day (1)	24.2 NO_3(1) & 12 Urea(3)	(2)	515 (± 87.9)	(Lindau et al. 1988b)
Crowley, LA (5)	(6_a)	4 – 19 day (1)	24.2 NO_3(1) & 12 Urea(3)	(2)	175 (± 107.7)	(Lindau et al. 1988b)
Crowley, LA (5)	6_b	18 day (1)	150 NH_4 (4)	(1)	232.0 - 336.1	(Reddy et al. 1989)
Crowley, LA (5)	6_a	18 day (1)	150 NH_4 (4)	(1)	23.8 - 50.6	(Reddy et al. 1989)

(**Appendix A.** continued)

Ecosystem [A]	Habitat [B]	Experiment Duration Location [C]	[^{15}N] Enrichment [D]	Technique [E]	Rate (µmol N m^{-2} h^{-1})	Reference
Crowley, LA (5)	6_b	10 day (1)	150 NO_3 (4)	(1)	874.6 - 2852.8	(Reddy et al. 1989)
Crowley, LA (5)	6_a	10 day (1)	150 NO_3 (4)	(1)	2.7 - 52.7	(Reddy et al. 1989)
Caernarvon Outfall Area (3)	(1_b)	2.25 hour (1)	70 NO_3 (1)	(5)	22.0 (± 3.1)	Current Study
Caernarvon Outfall Area (3)	(5)	2.25 hour (1)	70 NO_3 (1)	(5)	9.3 (± 4.6)	Current Study

Key ID:

A: 1= Barataria Basin; 2= Atchafalaya Basin; 3= Breton Sound;
4= Mississippi / Red River Floodplain; 5= Rice Exp. Station;

B: 1_a= Saline benthic sediment, 1_b= Fresh benthic sediment, 1c= Brackish benthic sediment
2= Bald cypress / water tupelo swamp; 3= Bottomland hardwood forest; 4= Saltmarsh;
5= Freshwater marsh; 6_a= Crowley silt loam, 6_b= Crowley silt loam + rice seeds

C: 1= Laboratory; 2= Field study; 3= Lab/Greenhouse study

D: 1= uM; 2= ug g^{-1}; 3= g m^{-2}; 4= mg soil $column^{-1}$

E: 1= $^{15}N_2$ Gas Emission; 2= ^{15}N Dilution; 3= Static Chamber; 4= ^{15}N Recovery 5= ^{15}N IPT

APPENDIX B. DENITRIFICATION RATES ESTIMATED USING THE ACETYLENE INHIBITION TECHNIQUE

Appendix B. Denitrification rates estimated using the acetylene inhibition technique in several ecosystems throughout coastal Louisiana for the period 1981-2009. For superscripts see key ID below table. (Modified from Rivera-Monroy et al. 2009, in review).

Ecosystem [(A)]	Habitat [(B)]	Experiment Duration Location [(C)]	[N] Enrichment [(D)]	Rate ($\mu mol\ N\ m^{-2}\ h^{-1}$)	Reference
(1)	(1_a)	142 day (1)	100 NO_3 (1)	58.2 (± 9.5)	(Childs et al. 2002)
(1)	(1_a)	142 day (1)	100 NO_3 (1)	108.1 (± 13.8)	(Childs et al. 2002)
(1)	(1_a)	142 day (1)	100 NO_3 (1)	47.9 (± 6.9)	(Childs et al. 2002)
(1)	(1_a)	142 day (1)	100 NO_3 (1)	39.8 (± 14.5)	(Childs et al. 2002)
(1)	(1_a)	142 day (1)	100 NO_3 (1)	103.3 (± 14.5)	(Childs et al. 2002)
(1)	(1_a)	142 day (1)	100 NO_3 (1)	69.3 (± 12.6)	(Childs et al. 2002)
(1)	(1_a)	142 day (1)	100 NO_3 (1)	63.1 (± 9.6)	(Childs et al. 2002)
(1)	(1_a)	1 day (1)	10 NO_3 (2)	44.6–148.7	(Delaune et al. 2005a)
(2)	(2)	3 hour (1)	Background	29.0–89.2	(Boustany et al. 1997)
(2)	(2)	3 hour (1)	3.0 NO_3 (1)	89.2–416.5	(Boustany et al. 1997)
(2)	(2)	3 hour (1)	3000 NO_3 (1)	59.5–1338.6	(Boustany et al. 1997)
Big Mar (3)	(1_b)	11 day (1)	Background	0.0 (±0.0)–2.8 (±0.5)	(Delaune and Jugsujinda 2003b)
Big Mar (3)	(1_b)	11 day (1)	1.75 NO_3 (3)	13.7 (±6.9)–199.5 (±53.3)	(Delaune and Jugsujinda 2003b)

(**Appendix B** continued)

Ecosystem [A]	Habitat [B]	Experiment Duration Location [C]	[N] Enrichment [D]	Rate (µmol N m^{-2} h^{-1})	Reference
Big Mar (3)	(1_b)	11 day (1)	3.5 NO_3 (3)	41.9 (±2.2)–349.8 (±6.5)	(Delaune and Jugsujinda 2003b)
(2)	(2)	8, 22, 30° C (1)	Background	0.18–14.2	(Lindau et al. 2008b)
(2)	(2)	65 day @ 8° C (1)	1613 NO_3 (1)	0.18–77.2	(Lindau et al. 2008b)
(2)	(2)	32 day @ 22° C (1)	1613 NO_3 (1)	0.18–163.6	(Lindau et al. 2008b)
(2)	(2)	17 day @ 30° C (1)	1613 NO_3 (1)	0.18–289.6	(Lindau et al. 2008b)
Four League Bay (2)	(1_a / 1_b)	12 hour (1)	25 & 50 NO_3 (1)	17.1	(Smith et al. 1985)
Four League Bay (2)	(3)	12 hour (1)	25 & 50 NO_3 (1)	13.9	(Smith et al. 1985)
Crowley, LA (4)	(5_a)	31 day (3)	9 Urea (3)	0.51 - 6.1	(Smith and Delaune 1984)
Crowley, LA (4)	(5_b)	31 day (3)	9 Urea (3)	0.22 - 7.2	(Smith and Delaune 1984)
Crowley, LA (4)	(5_a)	30 day (3)	9 Urea (3)	0.36 - 2.6	(Smith and Delaune 1984)
Crowley, LA (4)	(5_b)	30 day (3)	9 Urea (3)	0.71 - 2.9	(Smith and Delaune 1984)
Davis Pond (5)	(4)	7 day (1)	0.0 – 32.3 NO_3 (1)	5.7–274.9	(Gardner 2008)
Davis Pond (5)	(4)	24 hour (2)	16.1 NO_3 (1)	131.5	(Gardner 2008)
Lake Cataouatche (5)	(1_b)	24 day (1)	Background	0.2 (± 0.1)–2.0 (±0.4)	(Iwai 2002)
Lake Cataouatche (5)	(1_b)	24 day (1)	806 NO_3 (1)	10.7 (± 9.2)–280.1 (± 44.6)	(Iwai 2002)

(**Appendix B** continued)

Ecosystem [A]	Habitat [B]	Experiment Duration Location [C]	[N] Enrichment [D]	Rate (µmol N m^{-2} h^{-1})	Reference
Lac Des Allemands (5)	(1_b)	16 week (1)	50 NH_4 (2)	1.0 - 367.6	(Smith and Delaune 1983b)
Airplane Lake (5)	(1_a)	16 week (1)	50 NH_4 (2)	0.2 - 47.0	(Smith and Delaune 1983b)
Lake Cataouatche (5)	(1_b)	8.2 day (1)	Background	9.8	(Miao et al. 2006)
Lake Cataouatche (5)	(1_b)	8.2 day (1)	142.8 (1)	19.9	(Miao et al. 2006)
Lake Cataouatche (5)	(1_b)	8.2 day (1)	1428.6 (1)	137.9	(Miao et al. 2006)
Lake Cataouatche (5)	(1_b)	8.2 day (1)	2857.1 (1)	241.8	(Miao et al. 2006)
Davis Pond (5)	(4)	(1)	142.8 - 285.6 NO_3 (1)	92 (±29.7)–214 (±62.5)	(Delaune et al. 2005b)
Lake Cataouatche (5)	(1_b)	16 day (1)	Background	0.65	(Lindau et al. 2009)
Lake Cataouatche (5)	(1_b)	24 day (1)	806 NO_3	185.4 (±31.3)	(Lindau et al. 2009)

Key ID:
A: 1= Offshore; 2= Atchafalaya Basin; 3= Breton Sound; 4= Rice Exp. Station; 5= Barataria Basin; 6= Mississippi / Red River Floodplain
B: 1_a= Saline benthic sediment, 1_b= Fresh benthic sediment; 2= Bald cypress / water tupelo swamp; 3= Saltmarsh; 4= Freshwater marsh; 5_a= Crowley silt loam, 5_b= Crowley silt loam + rice seeds
C: 1= Laboratory; 2= Field study; 3= Lab/Greenhouse study
D: 1= uM; 2= ug g^{-1}; 3= g m^{-2}

APPENDIX C. DENITRIFICATION RATES ESTIMATED USING INDIRECT TECHNIQUES

Appendix C. Denitrification rates estimated using indirect techniques in several ecosystems throughout coastal Louisiana for the period 1981-2009. For superscripts see key ID below table, N/A = not included in study. (Modified from Rivera-Monroy et al. 2009 in review).

Ecosystem (A)	Habitat (B)	Experiment Duration Location (C)	[N] Enrichment (D)	Technique (E)	Rate (µmol N m^{-2} h^{-1})	Reference
(1)	(2)	10 day (1)	Background	(1)	29.8	(Smith and Delaune 1983a)
(1)	(2)	10 day (1)	0.05 & 1.2 NH_4 (2)	(1)	44.6 - 2157.7	(Smith and Delaune 1983a)
(1)	(2)	10 day (1)	Background	(1)	163.7	(Smith and Delaune 1983a)
(1)	(2)	10 day (1)	0.05 & 1.5 NH_4 (2)	(1)	163.7 - 1116.1	(Smith and Delaune 1983a)
Lac Des Allemands (1)	(4)	2 year (2)	N/A	(1)	100.4	(Smith et al. 1983)
Lac Des Allemands (1)	(1_b)	2 year (2)	N/A	(1)	62.9	(Smith et al. 1983)
West of Bayou Perot (1)	(2)	2 year (2)	N/A	(1)	87.1	(Smith et al. 1983)
West of Bayou Perot (1)	(1_c)	2 year (2)	N/A	(1)	38.2	(Smith et al. 1983)
East of Leville, LA (1)	(3)	2 year (2)	N/A	(1)	56.3	(Smith et al. 1983)
East of Leville, LA (1)	(1_a)	2 year (2)	N/A	(1)	87.1	(Smith et al. 1983)

(**Appendix C** continued)

Ecosystem (A)	Habitat (B)	Experiment Duration Location (C)	[N] Enrichment (D)	Technique (E)	Rate (µmol N m^{-2} h^{-1})	Reference
Baton Rouge, LA (2)	(5)	3 day (1)	Secondary Treated Wastewater / 554.4 NH_4 (1)	(1)	67.3 - 269.1	(Smith et al. 1981)
Baton Rouge, LA (2)	(5)	3 day (1)	Secondary Treated Wastewater / 554.4 NH_4 (1)	(1)	751.1 - 1268.3	(Smith et al. 1981)
Davis Pond (1)	(3)	(1)	32.3 – 64.5 NO_3 (1)	(2)	241.0 (±110.1)	(Delaune et al. 2005b)
Davis Pond (1)	(4)	(1)	N/A	(3)	190.4	(Delaune et al. 2005b)
Davis Pond (1)	(4)	7 day (1)	0.0 – 32.3 NO_3 (1)	(2)	130.9 - 407.5 (±32.7 / 71.4)	(Gardner 2008)
(3)	(1_a)	(1)	N/A	(4)	21 – 44	(Gardner et al. 1993)

Key ID:
A: 1= Barataria Basin; 2= USDA Ben Hur Research Farm; 3= Offshore;
B: 1_a= Saline benthic sediment, 1_b= Fresh benthic sediment, 1_c= Brackish benthic sediment; 2= Brackish Marsh; 3= Saltmarsh; 4= Freshwater marsh; 5= Mhoon silt loam
C: 1= Laboratory; 2= Field study; 3= Lab/Greenhouse study
D: 1= uM; 2= g m^{-2}
E: 1= N_2O emission; 2= NO_3 disappearance; 3= Mass balance; 4= Stoichiometric

APPENDIX D. SUMMARY OF DENITRIFICATION RATES FROM THE CURRENT STUDY

Appendix D. Summary of denitrification rates estimated using ^{15}N isotope pairing technique in upper Breton Sound, Louisiana for the current study period November 2006-April 2008. Direct denitrification value is from D_{15} and coupled nitrification-denitrification value is from D_n. For * and superscript definition see text at end of table

Habitat	Location	Date Sampled	Incubation Water Temp. (°C) [(A)]	Ambient NO_3^- (µM)	$^{15}NO_3^-$ Incubation (µM)	Direct Denitrification Rate (µmol N m^{-2} h^{-1})	Coupled Denitrification Rate (µmol N m^{-2} h^{-1})
Benthic	Near-Field	November 2006	22 (1)	48.3	54.5 *	0.26	57.7
Benthic	Near-Field	November 2006	22 (1)	48.3	73.6 *	2.3	281.8
Benthic	Near-Field	November 2006	22 (1)	48.3	110.5 *	0.93	31.2
Benthic	Near-Field	November 2006	22 (1)	48.3	151.4 *	2.8	55.4
Marsh	Far-Field	February 2007	22 (1)	101.1	7.9 *	0.10	1.5
Marsh	Far-Field	February 2007	22 (1)	101.1	69.5 *	0.22	5.0
Marsh	Far-Field	February 2007	22 (1)	101.1	101.6 *	0.16	5.0
Marsh	Near-Field	February 2007	22 (1)	101.1	7.9 *	0.08	2.4
Marsh	Near-Field	February 2007	22 (1)	101.1	69.5 *	0.86	44.5
Marsh	Near-Field	February 2007	22 (1)	101.1	101.6 *	0.47	9.1
Benthic	Near-Field	March 2007	22 (1)	135.7	9.1 *	3.5	1633.7
Benthic	Near-Field	March 2007	22 (1)	135.7	74.7 *	10.3	911.4
Benthic	Near-Field	March 2007	22 (1)	135.7	106.5 *	13.5	745.7
Benthic	Near-Field	April 2007	10 – 12 (1)	150.6	13.3 *	1.7	84.4
Benthic	Near-Field	April 2007	10 – 12 (1)	150.6	74.0 *	2.1	104.9
Benthic	Near-Field	April 2007	10 – 12 (1)	150.6	110.0 *	2.9	157.7
Benthic	Far-Field	July 2007	22 (1)	134.5	7.9	0.82	22.1
Benthic	Far-Field	July 2007	22 (1)	134.5	71.5	2.0	25.0
Benthic	Far-Field	July 2007	22 (1)	134.5	84.7	2.4	24.0

(**Appendix D** continued)

Habitat	Location	Date Sampled	Incubation Water Temp. (°C) [A]	Ambient NO_3^- (µM)	$^{15}NO_3^-$ Incubation (µM)	Direct Denitrification Rate (µmol N m^{-2} h^{-1})	Coupled Denitrification Rate (µmol N m^{-2} h^{-1})
Benthic	Far-Field	July 2007	8 (2)	134.5	71.5	0.75	7.2
Marsh	Far-Field	July 2007	22 (2)	31.1	62.5	0.67	8.4
Marsh	Far-Field	July 2007	8 (2)	31.1	62.5	0.19	0.83
Marsh	Near-Field	July 2007	22 (2)	63.1	51.9	0.39	0.75
Marsh	Near-Field	July 2007	8 (2)	63.1	51.9	0.74	2.3
Benthic	Near-Field	August 2007	22 (2)	80.3	71.5	1.0	3.9
Benthic	Near-Field	August 2007	8 (2)	80.3	71.5	0.45	1.4
Benthic	Near-Field	February 2008	22 (1)	161.6	31.1	0.30	5.7
Benthic	Near-Field	February 2008	22 (1)	161.6	71.3	1.0	32.8
Benthic	Near-Field	February 2008	22 (1)	161.6	205.7	3.5	22.3
Marsh	Near-Field	February 2008	22 (1)	161.6	31.1	0.31	0.55
Marsh	Near-Field	February 2008	22 (1)	161.6	71.3	0.70	1.5
Marsh	Near-Field	February 2008	22 (1)	161.6	205.7	0.60	1.6
Benthic	Far-Field	February 2008	22 (2)	147.2	74.7	0.94	14.3
Benthic	Far-Field	February 2008	8 (2)	147.2	74.7	1.0	8.3
Marsh	Far-Field	February 2008	22 (2)	112.9	72.4	0.38	0.28
Marsh	Far-Field	February 2008	8 (2)	112.9	72.4	0.39	0.93
Marsh	Near-Field	February 2008	22 (2)	112.9	72.4	1.0	3.0
Marsh	Near-Field	February 2008	8 (2)	112.9	72.4	0.48	1.6
Benthic	Near-Field	March 2008	22 (2)	121.3	76.8	0.84	23.0
Benthic	Near-Field	March 2008	8 (2)	121.3	76.8	0.33	15.0
Benthic	Far-Field	April 2008	22 (2)	94.1	78.1	2.2	14.6
Benthic	Far-Field	April 2008	8 (2)	94.1	78.1	0.67	7.1

(**Appendix D** continued)

Habitat	Location	Date Sampled	Incubation Water Temp. (°C) [A]	Ambient NO_3^- (µM)	$^{15}NO_3^-$ Incubation (µM)	Direct Denitrification Rate (µmol N m^{-2} h^{-1})	Coupled Denitrification Rate (µmol N m^{-2} h^{-1})
Benthic	Near-Field	April 2008	22 (2)	94.1	78.1	0.86	16.2
Benthic	Near-Field	April 2008	8 (2)	94.1	78.1	0.59	3.8
Marsh	Far-Field	April 2008	22 (2)	35.9	76.3	3.2	5.5
Marsh	Far-Field	April 2008	8 (2)	35.9	76.3	1.6	2.3
Marsh	Near-Field	April 2008	22 (2)	35.9	76.3	2.8	4.2
Marsh	Near-Field	April 2008	8 (2)	35.9	76.3	0.51	0.97

***** indicates the $^{15}NO_3^-$ incubation concentration was a mixture of $^{14}NO_3^-$ and $^{15}NO_3^-$. Mississippi River water was collected from Big Mar Lake in upper Breton Sound which contained $^{14}NO_3^-$ and was the solution $^{15}NO_3^-$ was added too prior to experimentation. The concentration presented is the total ($^{14}NO_3^-$ and $^{15}NO_3^-$) incubation concentration applied in the experiment. No * indicates incubation concentration contained only $^{15}NO_3^-$.
The superscript [A] is used to identify the type of experiment (i.e. IPT assumption or water temperature) with (1) = IPT assumption experiment and (2) = water temperature experiment.

APPENDIX E. SAMPLE ISOTOPE PAIRING TECHNIQUE CALCULATION

Appendix E. Sample calculations for estimating denitrification rates using the isotope pairing technique. Example is from Benthic Near-Field location, incubated at 22 °C and sampled on March 1, 2008.

1. Below is an example of the data output obtained from the Europa Scientific 20/20 isotope ratio mass spectrometer.

Site	Treatment [$^{15}NO_3^-$] µM	Time (h)	Rep.	Delta Ref.	Correct 2/1	Correct 3/1	Raw 2/1	Raw 3/1
B-NF	0	0.25	A	-0.1680	0.007352	1.365E-05	0.006992	3.90E-04
B-NF	0	0.75	A	-0.2306	0.007351	1.362E-05	0.006993	3.88E-04
B-NF	0	1.5	A	-0.3776	0.007350	1.359E-05	0.006993	3.86E-04
B-NF	0	2.25	A	-0.3333	0.007350	1.356E-05	0.006995	3.84E-04
B-NF	0	0.25	B	-0.1992	0.007351	1.370E-05	0.006992	3.91E-04
B-NF	0	0.75	B	-0.2802	0.007351	1.365E-05	0.006993	3.89E-04
B-NF	0	1.5	B	-0.3190	0.007351	1.357E-05	0.006994	3.86E-04
B-NF	0	2.25	B	-0.3350	0.007350	1.355E-05	0.006995	3.83E-04
B-NF	70	0.25	A	0.1220	0.007354	1.369E-05	0.006995	3.91E-04
B-NF	70	0.75	A	0.6399	0.007358	1.372E-05	0.007000	3.90E-04
B-NF	70	1.5	A	1.2313	0.007362	1.367E-05	0.007006	3.88E-04
B-NF	70	2.25	A	1.6371	0.007365	1.387E-05	0.007009	3.92E-04
B-NF	70	0.25	B	0.0319	0.007353	1.371E-05	0.006995	3.91E-04
B-NF	70	0.75	B	0.6355	0.007358	1.377E-05	0.007000	3.92E-04
B-NF	70	1.5	B	1.0639	0.007361	1.370E-05	0.007005	3.88E-04
B-NF	70	2.25	B	1.9602	0.007367	1.381E-05	0.007012	3.90E-04

NOTE: In the above experiment all $^{14}NO_3^-$ was removed from the water column prior to $^{15}NO_3^-$ addition, and therefore, the $^{15}NO_3^-$ was not diluted. Therefore D_{15} represents direct denitrification of $^{15}NO_3^-$, and D_n represents coupled nitrification-denitrification (Fig. 2).

2. After obtaining the sample data output from the mass spectrometer and sorting it by time, treatment and replicate, the mean background (control or zero treatment) [$^{29}N_2$] / [$^{28}N_2$] and [$^{30}N_2$] / [$^{28}N_2$] ratio is obtained for each time series in the zero treatment, "control" cores.

Using the correct 2/1 ([$^{29}N_2$] / [$^{28}N_2$]) and correct 3/1 ([$^{30}N_2$] / [$^{28}N_2$]) ratio from the control (zero) treatment and the initial time (0.25 h), the mean background is calculated as follows:

Mean background [$^{29}N_2$] / [$^{28}N_2$] = (0.00735 + 0.00735) / 2 = 0.00735
Mean background [$^{30}N_2$] / [$^{28}N_2$] = (1.37E-05 + 1.37E-05) / 2 = 1.37E-05

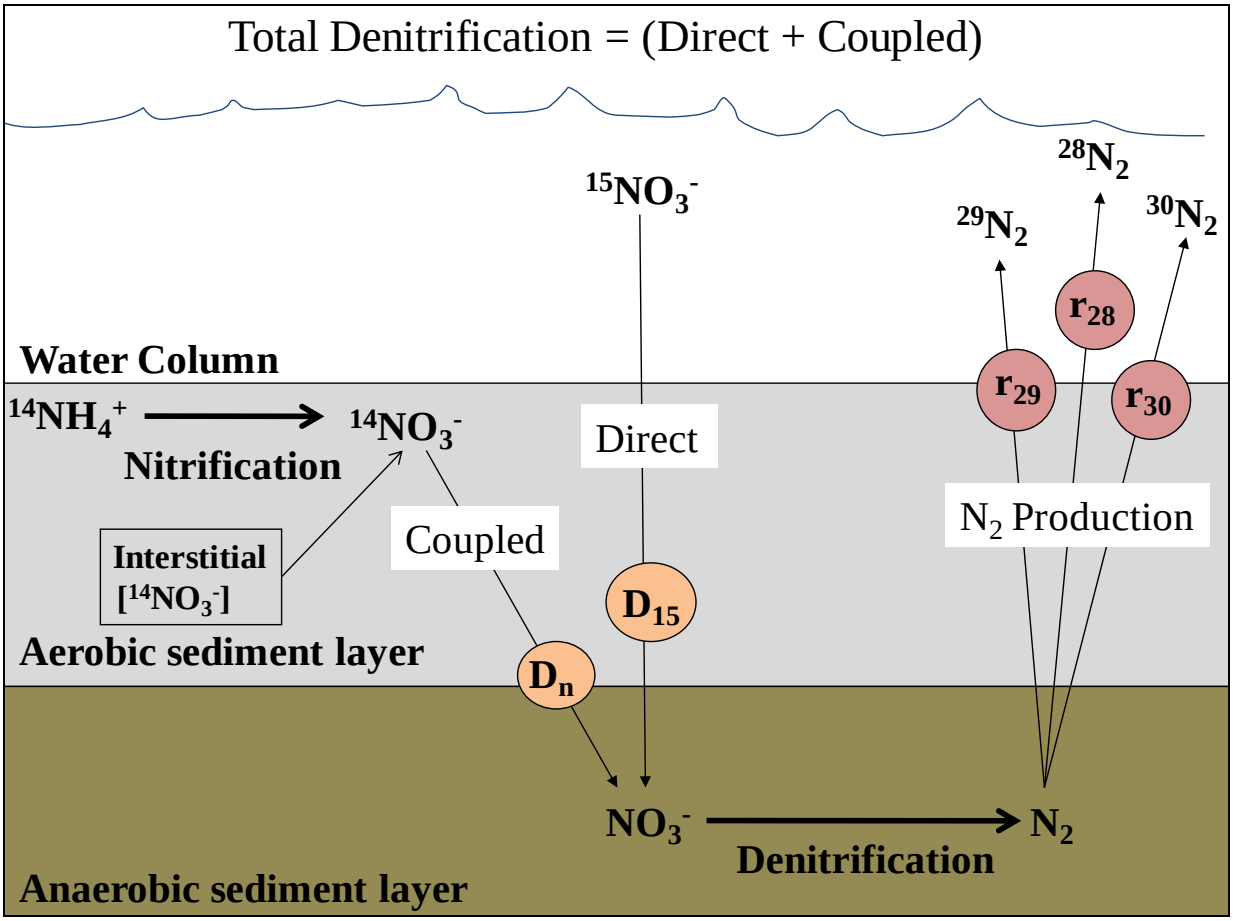

Fig. 2 Isotope pairing technique conceptual model applied during current study. Diagram illustrates contribution of ^{15}N and ^{14}N atoms via direct and coupled denitrification to labeled $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$ products (after Steingruber et al. 2001).

3. After calculating the mean background ratio from the control treatments for each time series (0.25-2.25), the background ratio is subtracted from the ratio obtained using the 70 µM treatments to obtain "excess [$^{29}N_2$] / [$^{28}N_2$] and [$^{30}N_2$] / [$^{28}N_2$]" for each time series and replicate (A & B). Continuing from the calculation above:

Excess [$^{29}N_2$] / [$^{28}N_2$] = 0.00735 – 0.00735 = 2.25E-06
Excess [$^{30}N_2$] / [$^{28}N_2$] = 1.37E-05 – 1.37E-05 = 1.38E-08

4. Once the "excess" ratios have been calculated for each time series (0.25-2.25) and replicate (A & B) we then calculate the concentration of [$^{28}N_2$] (mol/l) using an empirical formula which incorporates experimental conditions (i.e. incubation temperature and salinity) and multiple N_2 solubility coefficients (Weiss 1970; Hamme and Emerson 2004). For this particular experiment the [$^{28}N_2$] (mol/l) concentration was calculated based on a temperature of 22 °C and a salinity of zero ppt.

$[^{28}N_2]$ (mol/l) = 0.000519

5. Now we calculate the [$^{29}N_2$] and [$^{30}N_2$] (mol/l) concentrations from the incubated sediment cores for each time series and replicate using the [$^{28}N_2$] (mol/l). Continuing from the calculations above:

$[^{29}N_2]$ (mol/l) = 2.25E-06 • 0.000519 = 1.166E-09
$[^{30}N_2]$ (mol/l) = 1.38E-08 • 0.000519 = 7.165E-12

6. Next we calculate the [$^{29}N_2$] and [$^{30}N_2$] production rates (r_{29}) and (r_{30}) (mol N_2 m^{-2} h^{-1}), from the 70 µM incubation treatment. To do this we perform a linear regression using both replicates (A & B) from the [$^{29}N_2$] and [$^{30}N_2$] (mol/l) concentration over the entire time series (0.25, 0.75, 1.5 and 2.25 h). This gives us a slope (m_{29}) or (m_{30}) of the linear regression line to be used in the following equation:

$$R_{29} = m_{29}/A \bullet (V_w + \Phi V_s)$$

Where, A is the incubated sediment surface area (m^2), V_w is the incubated water volume (L), Φ is the sediment porosity and V_s is the incubated sediment volume (L). So, for the current 70 µM incubation treatment from the experiment above we calculate r_{29} and r_{30} as follows:

r_{29} = (3.489E-09 / 0.0003) • (0.035 + (0.78 • 0.0069)) = 4.033E-07
r_{30} = (6.342E-11 / 0.0003) • (0.035 + (0.78 • 0.0069)) = 7.331E-09

Once the production rates [$^{29}N_2$] (r_{29}) and [$^{30}N_2$] (r_{30}) are calculated we convert them from (mol N_2 m^{-2} h^{-1}) to (µmol N_2 m^{-2} h^{-1}) as follows:

r_{29} = 4.033E-07 • 1,000,000 = 0.403
r_{30} = 7.331E-09 • 1,000,000 = 0.0073

7. Once the N_2 production rates are obtained, we calculate denitrification rates from $^{15}NO_3^-$ and $^{14}NO_3^-$ using the following formulas:

$$D_{15} = r_{29} + 2 \bullet r_{30}$$
$$D_{14} = D_{15} (r_{29}/2 \bullet r_{30})$$

Using the (r_{29}) and (r_{30}) (μmol N_2 m^{-2} h^{-1}) production rates from above we obtain the following denitrification rates:

$D_{15} = 0.403 + (2 \bullet 0.0073) = 0.418$ (μmol N_2 m^{-2} h^{-1})
$D_{14} = 0.418 \bullet (0.403/ (2 \bullet 0.0073)) = 11.50$ (μmol N_2 m^{-2} h^{-1})

8. The total denitrification within the sediment is obtained using the following formula:

$$D^{tot} = D_{14} + D_{15}$$

Using the D_{15} and D_{14} denitrification rates we obtain a total sediment denitrification rate:

$D^{tot} = 0.418 + 11.50 = 11.91$ (μmol N_2 m^{-2} h^{-1})

9. Finally, to calculate the coupled nitrification-denitrification rate we use the following formula:

$$D_n = D^{tot} - D_{15}$$

And, coupled nitrification-denitrification is calculated for the above experiment as follows:

$D_n = 11.91 - 0.42 = 11.49$ (μmol N_2 m^{-2} h^{-1})

10. Therefore, the direct and coupled nitrification-denitrification rate for the above experiment is:

$D_{15} = 0.418$ (μmol N_2 m^{-2} h^{-1})
$D_n = 11.49$ (μmol N_2 m^{-2} h^{-1})

11. Finally, we convert the direct and coupled denitrification rates from (μmol N_2 m^{-2} h^{-1}) to (μmol N m^{-2} h^{-1}) by multiplying by two:

$D_{15} = 0.418 \bullet 2 = 0.84$ (μmol N m^{-2} h^{-1})
$D_n = 11.49 \bullet 2 = 22.97$ (μmol N m^{-2} h^{-1})

VITA

Peter L. Lenaker is a native of Antigo, Wisconsin. He was born in August 1981, to Ronald and the late Sylvia Lenaker and has three elder brothers; Steve, Mark and Paul. He graduated from Antigo Senior High School in May 1999. Upon graduation he moved to Fairbanks, Alaska, to play Junior A hockey for the Fairbanks Icedogs. After a short stint in Alaska he began college at the University of Wisconsin-Marathon County. After one year enrolled at the University of Wisconsin he enrolled in AmeriCorps and began a year of community service in Seattle, Washington, with the non-profit environmental organization Earthcorp. While at Earthcorp his appreciation for nature, ecological processes and wetlands motivated him to return to school and purse an environmental discipline. After his year of community service he enrolled at North Seattle Community College where he graduated with an Associate of Arts degree. After North Seattle Community College Peter attended Western Washington University-Huxley College of the Environment in Bellingham, Washington, and graduated with a Bachelor of Science in Environmental Science degree in June 2006. Peter also completed minors in environmental policy and chemistry while attending Western Washington University. Two weeks after graduation his mother, Sylvia, passed away. This motivated Peter to continue his education at Louisiana State University in Baton Rouge, Louisiana, under the guidance of Dr. Robert R. Twilley. During his master's education Peter married his high school sweetheart, Jacqueline Melzer on June 7, 2008. Nine months later Peter and Jackie were blessed with the birth of a healthy baby girl, Willa Sylvia Lenaker, on March 11, 2009. Peter began work for Dr. Twilley in September 2006 as a Research Associate, switched to a graduate assistant and full-time student in January 2007 and concluded his degree in December 2009.